Australian and New Zealand College of Veterinary Scientists

Dermatology Chapter
Science Week Proceedings

WHEN NATURE GOES WRONG
Understanding and Treatment of Sterile Immune Mediated Dermatological Diseases
July 6th-7th 2018, Gold Coast
THANK YOU

The Dermatology Chapter of the Australian and New Zealand College of Veterinary Scientists would like to thank Hills for their generous sponsorship in 2018.
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The 14th Annual Dermatology Chapter Meeting of the Australian New Zealand College of Veterinary Scientists.

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Biographies

Prof. Petra Bizikova, DVM, PhD, dipACVD, DipECVD

Petra Bizikova graduated at the University of Veterinary Medicine in Kosice [Koshice], Slovakia in 2001 with honors. She subsequently joined one of the largest private veterinary clinics in Slovakia at that time, where she worked for four years and focused primarily on internal medicine, cytology and dermatology.

A personal quest to improve her skills and medical knowledge in veterinary dermatology led her to multiple, self-funded, externships at private referral dermatology clinics and university teaching hospitals in Europe and in the United States. A fortunate trip to North Carolina State University gave her the chance to start an internship followed by a residency in veterinary dermatology and immunology PhD. She became boarded by both American and European Colleges of Veterinary Dermatology in 2010 and defended her PhD in 2012. As a result of her PhD, she was able to identify the major target autoantigen in canine pemphigus foliaceus. After finishing PhD, she then joined the Dermatology Faculty of the College of Veterinary Medicine in 2012 as a clinician-scientist with a main interest in autoimmunity and atopic dermatitis. She is also managing equine dermatology at the NCSU.

Prof. Peter B. Hill BVSc (Hons) PhD DVD DipACVD DipECVD MANCVS

Peter Hill graduated from the University of Liverpool in 1986 and spent five years in small animal practice before moving to the USA to undertake a residency in veterinary dermatology at the University of Wisconsin-Madison. In 1993, he returned to the UK and completed a PhD on mast cell biology at the Royal (Dick) School of Veterinary Studies, Edinburgh, followed by a further eight years as lecturer and then senior lecturer in veterinary dermatology. In 2005, he took up the position of senior lecturer in veterinary dermatology at the University of Bristol, combining clinical and teaching responsibilities with an active research program.

In 2009, he emigrated to Australia and worked in private referral practice before taking up his current post at the University of Adelaide. He is currently Professor of Veterinary Dermatology and Immunology and Head of Department of Companion Animal Health. Dr. Hill holds the RCVS Diploma in Veterinary Dermatology and is a Diplomate of both the American and European Colleges of Veterinary Dermatology. He is the author of over 100 scientific publications and book chapters and has given over 250 invited talks and presentations around the world.
Dr. Rachel Peters
Post Doctoral Research Fellow

Dr Rachel Peters is an epidemiologist with a special interest in food allergy and allergic diseases. She completed a Master of Public Health (epidemiology and biostatistics) at the University of Melbourne in 2011, and attained her PhD in 2014 at the University of Melbourne and Murdoch Children's Research Institute. Her thesis examined predictors and phenotypes of food allergy and the development of tolerance in childhood.

Dr Peters currently works at Murdoch Children's Research Institute and is a member of the NHMRC-funded Centre of Research Excellence, the Centre for Food and Allergy Research. Her current research areas include the epidemiology of food allergy, identifying biomarkers for the diagnosis and prognosis of food allergy, and elucidating the role that food allergy plays in the allergic march of childhood.

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Dr. Evie Knight
BVSc, MANZCVS (Small Animal Medicine), FANZCVS, DipACVD, Registered Specialist in Veterinary Dermatology

Evie graduated from the University of Melbourne in 2010. After graduation she undertook a rotating internship in small animal medicine and surgery at Queensland Veterinary Specialists and Pet Emergency in Brisbane. Following completion of the internship, she commenced a dermatology internship at Dermatology for Animals.

Evie was then offered a residency position in veterinary dermatology, training under both the Australian and American residency programs, with time spent in externships in both Australia and the USA.

Evie is a member of the Australian College of Veterinary Scientists (MANZCVS) by examination in small animal medicine, and is a Fellow of the Australian and New Zealand College of Veterinary Scientists (FANZCVS) by examination in veterinary dermatology. She is also a diplomate of the American College of Veterinary Dermatology (DipACVD) and is a registered specialist. She lectures frequently at local and national conferences on a range of topics.

Dr. Allan Bell

Allan graduated from Massey University in 1967 and worked in companion animal practice until the mid 80's. He became a member of the Australian College in Canine medicine in 1981 and attained a Fellowship in Dermatology in 1995. He is a registered specialist and before his recent retirement, had a referral dermatology practice based in Auckland. He is first author of 6 papers published in referred journals and was an invited speaker at the 1999 World Small Animal Association Congress. He runs a small deer farm and enjoys sailing.
Desmosome autoimmunity in dogs, cats and horses

Petra Bizikova, MVDr, PhD, dipACVD, dipECVD
North Carolina State University, College of Veterinary Medicine, Department of Clinical Sciences, Raleigh, NC, USA

Mechanism of blister formation:
An intact skin is a critically important organ that functions as a first-line defense mechanism against physical and chemical damage. Its integrity is dependent on complex structures maintaining cell-cell and cell-matrix adhesion.1,2 Several autoimmune skin diseases disrupting this cohesion have been recognized. The mechanism by which this adhesion is disrupted varies depending on the type of disease.

a) Disruption of basement membrane adhesion – subepidermal blister formation due to dermo-epidermal separation (bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), epidermolysis bullosa acquisita (EBA), linear IgA disease (LAD), mixed autoimmune subepidermal blistering disease (mixed AISBD))
b) Disruption of keratinocyte adhesion – intraepidermal blister formation due to desmosome dissociation (pemphigus foliaceus (PF), pemphigus vegetans (PVeg), pemphigus vulgaris (PV), paraneoplastic pemphigus (PNP))
c) Keratinocyte injury and interface dermatitis – keratinocyte-targeting diseases causing disruption of epidermal cohesion (cutaneous lupus, erythema multiforme complex, etc.)

Pemphigus diseases – a desmosome autoimmunity

Introduction
Veterinarians have been aware of the existence of a naturally occurring pemphigus in domestic animals for decades.3,4 Canine pemphigus, like in people, encompasses four variants: pemphigus foliaceus (PF), pemphigus vulgaris (PV), pemphigus vegetans (PVeg) and paraneoplastic pemphigus (PNP).5 Pemphigus erythematosus, a facial-restricted variant of pemphigus foliaceus, is an entity characterized by subcorneal acantholysis accompanied by a lichenoid interface dermatitis.5 Pemphigus foliaceus is the most common pemphigus variant in small animal and horses, while PV, the most common variant in people, is seen only rarely.5,6 The other pemphigus variants are even rarer with less than a handful of cases published in the literature over the past 30 years.

These two presentations will review desmosomal autoimmunity in dogs, cats and horses. The first hour will focus on canine PF, and, particularly, on following topics: i) Canine PF and autoantibodies; ii) “Atypical” PF – a diagnostic challenge; iii) Canine PF and treatment strategies. The following hour will review feline and equine PF, and, if time permits, deep forms of pemphigus in dogs, cats and horses. Currently recognized forms of pemphigus are summarized in Table 1.
# Table 1: Pemphigus diseases in domestic animals and people

<table>
<thead>
<tr>
<th>Disease</th>
<th>Affected Species</th>
<th>Over-represented Breeds</th>
<th>Age Predisposition</th>
<th>Characteristic skin lesions</th>
<th>Characteristic Lesion Distribution</th>
<th>Target Autoantigen</th>
<th>Histopathology</th>
<th>Human Counterpart (major antigen)</th>
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</thead>
<tbody>
<tr>
<td><strong>Superficial Pemphigus</strong></td>
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<tr>
<td>Pemphigus foliaceus (PF)</td>
<td>dog</td>
<td>chow-chows, akita</td>
<td>middle-aged (median: 6 years)</td>
<td>subcorneal pustule, eroded, crust and alopecia</td>
<td>DSG1 (major), DSG2</td>
<td>Linear</td>
<td>Subcorneal epidermal or follicular (parakeratotic) pemphigus, or pemphigus foliaceus (pemphigus may be present) containing acantholytic keratinocytes</td>
<td>PF (DSG1)</td>
</tr>
<tr>
<td></td>
<td>cat</td>
<td>not specified (most common)</td>
<td>middle-aged (median: 6 years)</td>
<td>pustules (pale in cats and horses), shallow erosions, thick crusts, scale, crust and alopecia</td>
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<tr>
<td></td>
<td>horse</td>
<td>not determined</td>
<td>median: 7 years</td>
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<td></td>
<td>small ruminants</td>
<td>not determined (median: 1.5 years)</td>
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<tr>
<td><strong>Deep Pemphigus</strong></td>
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<tr>
<td>Pemphigus trigonum (PT)</td>
<td>dog</td>
<td>German shepherd, collie</td>
<td>middle-aged (median: 6 years)</td>
<td>flaccid vesicle, deep erosions and ulcers</td>
<td>mucous and mucocutaneous keratin (oral cavity frequent) with or without skin involvement</td>
<td>DSG3, DSG5 (in mucocutaneous form)</td>
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<tr>
<td></td>
<td>cat</td>
<td>not determined</td>
<td>median: 5 years</td>
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<td>horse</td>
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<td>mare</td>
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<tr>
<td>Paraneoplastic Pemphigus</td>
<td>dog</td>
<td>not determined</td>
<td>not determined</td>
<td>flaccid vesicles, deep erosions and ulcers</td>
<td>mucous and mucocutaneous keratin with skin involvement</td>
<td>DSG3 + plakins (e.g. plakophilin, plakegamers)</td>
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<tr>
<td></td>
<td>cat</td>
<td>not determined</td>
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Abbreviations: nd - not determined (due to insufficient number of reported cases); DSG - desmoglein; DSC - desmocollin; major autoantigen - antigen recognized by more than 50% of affected animals.

## 1. Superficial pemphigus

### 1.a Pemphigus foliaceus (PF)

**Epidemiology, signalment, etiology**

Pemphigus foliaceus is the most common autoimmune skin disease recognized in dogs, cats, horses and small ruminants. While exact epidemiological data are not available for any of the species, the estimated incidence is about 3 cases per 1000 dogs referred for skin issues per year, and about 1 case per 100 cats and horses referred for skin issues per year. No prevalence data are known for small ruminants. There is no clear sex predilection apparent in dogs and horses, though in cats, females are marginally overrepresented. In dogs, Akitas and Chows appear to be at higher risk to develop PF. The median age of onset, if known, is listed in Table 1.

Pemphigus foliaceus is a complex disease with a myriad of potential triggering factors. While, the breed predispositions and the occurrence of PF in littermates described in dogs suggest the role of genetics, little is known about other triggers. Exceptions include UV-light and drugs. A group from Japan proposed sunlight as one of such triggers by demonstrating a worsening of clinical scores during the summer months in 10 out of 12 dogs with PF. Similar studies carried out in human patients with PF also demonstrated the ability of UVB light to induce acantholysis in an unaffected skin of tested subjects. Several case reports of drug-triggered PF in dogs and cats can be found in the literature, including the most recent cases of a drug-triggered PF in dogs following the administration of specific flea and tick preventative containing insecticides such as metaflumizone/amitraz (Promeris Duo; Pfizer), fipronil/amitraz/S-methoprene (Certifilc; Merial) or dinotefuran/pyriproxyfen/permethrin (Vectra 3D; Ceva Animal Health).

### Clinical signs

The characteristic lesion of PF is a subcorneal pustule, which rapidly progresses into an erosion, crust, scale-crust and alopecia. Crusting, scaling and alopecia are often the only lesion types seen in cats and horses. Flaccid vesicles and bullae, primary lesions typical for human PF, are not usually seen in our domestic animals. Pustules vary from small to large, and due to their ability to coalesce, they frequently exhibit irregular shapes. Additionally, they may organize into unique annular to polycyclic patterns described in dogs, cats and horses. The cyclical nature of the disease often leads to repeated pustule formation and to a subsequent formation of thick, multilayered crusts, which are the most common lesions found in all animals reported to suffer with PF. Alopecia is often present in
more severely affected areas, but the exfoliative erythroderma, a phenotype recognized in people, is rare in animals.\textsuperscript{7, 27, 28} Indeed, exfoliation and epidermal collarettes, as seen in exfoliative superficial pyoderma, are not typical for canine PF.

In most dogs, initial lesions appear on the face, particularly on the nasal planum, dorsal muzzle, eyelids and/or pinnae in a bilaterally symmetric pattern.\textsuperscript{27} The presence of this classic facial pattern in conjunction with acantholytic pustule formation is pathognomonic and the diagnosis of PF can be easily made without any reason for doubt. In contrast, subcorneal pustular dermatitis with acantholysis without the classic facial phenotype presents a diagnostic challenge (clinical and immunological features and its comparison to superficial pyoderma will be discussed during the presentation). Based on the largest case series, in dogs with the classic phenotype, skin lesions remain restricted to the face in about 16% of dogs, whereas in most dogs (66%) the disease becomes more generalized with trunk (58%), pinnae (51%) and dorsal muzzle (41%) being the most frequently affected sites.\textsuperscript{17} Dogs with PF often exhibit lesions on footpads (35%), which consist of pustules, erosions and crusts.\textsuperscript{17} With time, affected footpads become thick, hyperkeratotic with fissure formations. Pain is often seen in these cases. Additional clinical signs include pruritus, which is reported in about one third of affected dogs, and systemic signs (lethargy, fever, anorexia) usually associated with the severe generalized disease phenotype.\textsuperscript{7, 17, 27}

In case of the drug-triggered PF, caused by topical flea and tick preventatives, the initial lesion appears always in the area between the shoulders and/or along the spine - at the site of application of the topical product.\textsuperscript{24-26} In the majority of these dogs (70%), lesions progress to distant skin sites that mimic spontaneous PF (face, ears, nasal planum, dorsal muzzle, footpads).

In most cats, lesions often appear on the face (pinnae, eyelids and/or nasal planum/dorsal muzzle), and feet (claw folds and/or footpads). Involvement of the multiple claw folds and periareolar area are considered pathognomonic for PF, though the frequency of the latter sign varies from 7-20% based on the recent comprehensive literature review (Bizikova/Burrows; submitted). Pruritus that varies from mild to severe, is seen in about one third of affected cats and systemic signs such as lethargy and/or fever are reported in about half of the cases.

In horses, multifocal to generalized scaling, crusting and alopecia affecting the face (pinnae, muzzle, eyelids), extremities (distal limbs, coronary bands), neck and/or trunk are often observed. Pustules, like in cats, are rarely seen. Ventral edema and systemic signs (lethargy, fever, anorexia) are seen in about half of the patients.\textsuperscript{29, 30}

**Histopathology**

Histopathology of PF lesions is similar among dogs, cats and horses. A subcorneal to intragranular pustule containing individualized or clustered acantholytic keratinocytes is a characteristic lesion.\textsuperscript{7, 15, 27, 29-33} Pustules often span several hair follicles and may extend into infundibula. Neutrophils are the predominant inflammatory cell type; however, some cases exhibit a prominent eosinophil component. Neutrophils may encircle and cling to the acantholytic keratinocytes, a feature often seen in cytological samples.\textsuperscript{32} The degree of inflammation in the subcorneal and intragranular blisters in animal PF is markedly more prominent than that described in people with spontaneous PF.\textsuperscript{34} Due to the transient nature of the pustules, especially in cats and horses, and the cyclical nature of the disease, multilayered neutrophilic or eosinophilic crusts with ghost acantholytic cells may be the only histological finding in some cases.\textsuperscript{7, 29, 30, 33}

Finally, distinct histological differences between spontaneous canine PF and that triggered by the topical flea and tick preventatives have not been uncovered. However, epidermal necrosis in association with pustule formation and premature necrosis of acantholytic cells observed in dogs with drug-triggered PF due to the topical flea and tick preventatives could be an indicator of this etiopathogenesis.\textsuperscript{24, 26}

**Immunopathology**

Like in people, animal PF is believed to be an antibody-mediated autoimmune blistering skin disease targeting keratinocyte adhesion organelles called desmosomes.\textsuperscript{35} The deposition of antikeratinocyte IgG and rarely IgM, IgA and complement C3 in the lesional skin of PF-affected dogs was demonstrated more than three decades ago.\textsuperscript{15, 15, 15, 16, 16, 16, 27, 27, 36-40} Tissue-bound antikeratinocyte autoantibodies have been also found in other species, though it is not always clear if these autoantibody deposits were present in both lesional and non-lesional skin samples.\textsuperscript{8, 10, 15, 41}

Indeed, lesional skin from dogs with other dermatoses has been shown to contain antikeratinocyte autoantibody deposits.\textsuperscript{37, 38}

Circulating antikeratinocyte autoantibodies, predominantly IgG, were uncovered later in about 80% of dogs affected with PF.\textsuperscript{35, 42-44} Interestingly, low antikeratinocyte IgG titers were also detected in a high number of healthy dogs, but not in sera from dogs raised under specific-pathogen-free conditions.\textsuperscript{35} When different IgG subclasses were evaluated, antikeratinocyte IgG4 were detected in most canine PF sera, but only in rare sera from healthy dogs.\textsuperscript{35} Those detected in healthy canine sera were predominantly of IgG1 subclass.\textsuperscript{35} This observation resembled that seen in human endemic PF in the Limao Verde reservation in Brazil (Fogo Selvagem, FS), where many healthy individuals produce anti-desmoglein-1 (DSG1) IgG1 autoantibodies, while a strong IgG4 response is usually typical for PF-affected individuals.\textsuperscript{35} This immunological response in FS is believed to be a response to a noninfectious environmental antigen such as a salivary protein from a sand fly.\textsuperscript{35, 45} Similarly, the absence of antikeratinocyte IgG in dogs raised under specific-pathogen-free conditions would suggest that, a yet unknown, environmental trigger
could play role in development of PF in genetically susceptible dogs. Indeed, an older study suggested a possible role of fleas in eliciting PF based on the observation that the most common skin disease preceding or found concurrently with PF was flea allergy dermatitis. This conclusion could have been coincidental, since flea allergy dermatitis represented one of the most common skin disease in dogs at the time the manuscript was published. Further studies are needed to address this question. Circulating antikeratinocyte autoantibodies have been detected historically in horses with PF, though the same study failed to demonstrate antikeratinocyte IgG autoantibodies in cats with PF. This could be due to the low reliability of the indirect immunofluorescence (IF) testing at that time. Indeed, in our laboratory, up to 70% of sera from cats with PF had detectable antikeratinocyte IgG when feline and canine footpad and buccal mucosa were used as substrates (Levy, unpublished). However, like in dogs, lower titers of antikeratinocyte IgG were detected in about 20% of sera from healthy and allergic cats (Levy, unpublished).

Like in people, the pathogenicity of canine PF IgG antibodies was confirmed by passive transfer to neonatal mice. In these studies, transfer of IgG from PF-affected, but not healthy, dogs caused acantholysis and blister formation in the superficial epidermis of mice. The pathogenicity of circulating IgG antibodies in other animal species has not been demonstrated yet.

The major target autoantigen in canine PF differs from that recognized in people. Indeed, while the major autoantigen in human PF is DSG1, the autoantigen recognized by the majority of dogs with PF (spontaneous or drug-triggered) is desmocollin-1 (DSC1). Interestingly, anti-DSC1 autoreactivity in people appears to be mostly restricted to atypical PF variants in which a strong neutrophilic inflammation accompanies the blister formation, a feature similar to that of canine PF. Moreover, DSC1 is also recognized as a target autoantigen in people suffering with another neutrophil-rich dermatosis called subcorneal pustular dermatosis type IgA pemphigus. The major target autoantigen has not been uncovered in other animal species.

1. **Pemphigus erythematosus (PE)**

Pemphigus erythematosus is, even in people, a controversial clinical entity with lesions localized to the face. In veterinary medicine, PE has been associated with clinical phenotype in which face-predominant PF lesions (pustules, erosions and crusts), present in conjunction with discoid lupus erythematosus (DLE)-like lesions affecting the nasal planum (depigmentation, atrophy, erosions and ulcers). Histopathology reveals subcorneal to intragranular pustules with acantholysis along with a lichenoid interface dermatitis resembling DLE (reviewed in 7).

2. **Deep pemphigus**

2.a **Pemphigus vulgaris (PV)**

**Epidemiology and signalement**

In contrast to people, pemphigus vulgaris is considered to be one of the rarest autoimmune dermatoses in animals. It has been recognized in dogs, cats, and a horse. Because of the small number of described cases, breed, age and sex predilections cannot be reliably estimated in cats and horses. In dogs, it appears that German shepherds and collies are overrepresented compared to other reported breeds. This disease usually affects middle-aged to older dogs (median age of onset: 6 years) with almost half of the patients developing the disease after seven years of age. Males appear to be slightly overrepresented.

**Clinical signs**

Similarly to people, the primary lesion of animal PV is a flaccid vesicle rapidly progressing to an erosion. Further epithelial splitting beyond the preexisting erosion, even extending to a great distance (marginal Nikolskiy’s signs), can be elicited by pulling on the blister remnant. Crusts can develop over lesions at the mucocutaneous junctions or haired skin. Most commonly affected areas include mucosae/mucocutaneous junctions (oral cavity, nasal planum, lip margins, genitalia, anus and eyelids), and pinnae. Hypersalivation and halitosis are common signs in animals with oral lesions. Haired skin may or may not be involved. Claw shedding has been described in dogs with PV. Systemic signs such as lethargy or anorexia are often seen (about half of the reported dogs).

**Histopathology**

Histopathological features of animal PV are identical to those seen in people. Suprabasal acantholysis leads to a blister formation, which is usually free of any inflammatory cells. Acantholysis often extends into hair follicle infundibuli as well. Dermal inflammation is variable and it depends on the age of the lesion. The submucosal inflammation and dermal inflammation from mucocutaneous junctions is usually characterized by a superficial lymphoplasmacytic band-like infiltrate, which is considered a nonspecific inflammatory response seen in these anatomic locations.
Immunopathology

Direct and indirect IF reveal tissue-bound and circulating antikeratinocyte IgG in most affected dogs. Like in people, IgG4 is the most common subclass followed by IgG1. And, like in people, the major target autoantigen of canine PV is DSG3 with additional anti-DSG1 autoreactivity detected in some dogs with lesions affecting both mucosae and haired skin. Circulating antikeratinocyte IgG autoantibodies, including those targeting DSG3 specifically, are pathogenic as demonstrated by development of suprabasal blister formation after passive transfer to neonatal mice and by keratinocyte dissociation assays. In cats, direct IF revealed intercellular IgG and C3 deposits in 4/4 and 1/4 biopsy samples, respectively, but indirect IF was negative in all cats. In the horse with PV, direct and indirect IF revealed skin fixed and circulating antikeratinocyte IgG, respectively. IgM and IgA autoantibodies were not detected. The circulating IgG autoantibodies precipitated canine DSG3 and DSG1 recombinant proteins. Pathogenicity of the feline and equine PV-associated IgG autoantibodies has not been evaluated yet.

2.b Pemphigus vegetans (PVeg)

Epidemiology and signalment

Pemphigus vegetans, a variant of PV, is one of the rarest forms of pemphigus in animals and people. Only three published canine cases can be found in the literature; however, not all of them fulfill diagnostic criteria accepted in human medicine (i.e. PV-like suprabasal acantholysis with epidermal hyperplasia, papillomatosis and intraepidermal abscesses, which often exhibit high content of eosinophils, etc.). The three dogs were of different breeds (fox terrier, 14 years, female spayed; chow-chow, 7 years, female; greater Swiss mountain dog, 4 years, male). Because of the limited number of described cases and uncertainty of the actual diagnosis, breed, age and sex predilections cannot be determined.

Clinical signs

In people, two clinical forms of PVeg have been recognized. In the Neumann PVeg, patients develop flaccid blister and widespread erosions, which subsequently heal with excessive proliferation causing development of wart-like (verrucous) plaques. In the Hallopeau PVeg, patients develop crops of annular pustules on the basis of which verrucous plaques appear later. An overlap of both phenotypes, in which flaccid vesicles and pustules occur concurrently, has been described as well. P Veg plaques usually affect flexural areas (groin, axillae, inter-gluteal folds, umbilicus or interdigital folds). Mucosal lesions are commonly detected (88% based on a recent study) with oral and genital mucosae being the most often affected sites. In the three canine cases in the literature, only one dog exhibited mucosal lesions (oral cavity, prepuce and anus). In addition, this dog exhibited vesicles as well as pustules could on the abdomen, groin and feet. Pinnae, axillae and sternum exhibited verrucous, hyperkeratotic papules and plaques. The second case was described to have reddish-pink verrucous vegetations studded with pustules in axillae, groin, chest, abdomen and proximal extremities. The clinical picture and description (pustules, crusts and scales on the face, ears and trunk) of the last dog are more suggestive of PF.

Histopathology

Human PVeg, while being a variant of PV, exhibits unique histological features. Suprabasal acantholysis, a hallmark of PV, is often seen in PVeg, but may diminish with time, especially if extensive epidermal proliferation is present. The latter is characterized by papillomatous or verrucous proliferation of the epithelium. Intraepithelial microabscesses containing neutrophils and eosinophils with occasional apoptotic or necrotic keratinocytes are seen. Acantholysis is also observed. Similar histopathology was reported in the most recently published case of canine PVeg. None of the other two cases had histological evidence of suprabasal acantholysis; however, as this feature has been reported to diminish with time in people, this feature may not be considered a diagnostic criterion for the canine counterpart.

Immunopathology

Tissue-bound and circulating antikeratinocyte IgG were detected in the most recently published canine PVeg case. These antibodies were shown to precipitate recombinant canine DSG1 and DSC1, but not DSG3, which is the major target autoantigen in human PVeg. Interestingly, in people, IgG autoantibodies targeting DSG1, DSC1, DSC3 or periplakine are uncovered only occasionally. The reason for the lack of anti-DSG3 autoreactivity in this single canine PVeg case is unknown.
2.c Paraneoplastic pemphigus (PNP)

Epidemiology and signalment

In people, PNP is a rare autoimmune blistering skin disease developed in people with concurrent neoplasia. Hematologic-related neoplasms such as lymphoma, leukemia or thymoma are associated with 84% of human PNP cases. This disease is known to be associated with an extremely high rate of mortality (up to 90%) in people. Similarly, PNP is rarely seen in animals, and only three canine and one feline cases have been described so far (reviewed in ). All three dogs were of different breeds (boxer 11 years; bouvier 7 years; retriever 7 years), two were spayed females and one was castrated male. The cat was an 8-year-old female spayed Himalayan cat. Due to the small number of described cases, the information about the breed, age or sex predilection cannot be made.

Clinical signs

In people, clinical signs of PNP consist of painful mucocutaneous erosions resembling PV together with targetoid papules and plaques resembling erythema multiforme. Hemorrhagic stomatitis with lesions extending on the lips is considered unique for human PNP when compared to PV. Only rare cases will not exhibit mucosal lesions. In dogs, extensive erosions and ulcers affecting mucosae, mucocutaneous junctions, nasal planum and/or haired skin have been observed. Systemic signs (hyperthermia, depression) were reported in all three dogs and all dogs died as a result of the disease. On post mortem examination, a thymoma, a metastatic thymic lymphoma and an undifferentiated splenic sarcoma were identified. The single feline case exhibited extensive erosions and ulcers in axillae, ventral chest, caudal abdomen and medial thighs. Similar lesions affected concave pinnae. Mucosae or mucocutaneous junctions were not affected. The skin lesions appeared about three weeks after a surgical removal of thymoma. Interestingly, this cat also developed another autoimmune disease, myasthenia gravis, approximately two weeks after the surgery. Both autoimmune disease resolved with treatment and did not return once drugs were discontinued.

Histopathology

Human PNP exhibits variable histological features including suprabasal acantholysis (PV feature), scattered apoptotic keratinocytes and mononuclear interface dermatitis (resembling erythema multiforme or graft-versus-host disease), and even dermo-epidermal separation (resembling bullous pemphigoid). In the three dogs and the cat, suprabasal acantholysis and apoptotic keratinocytes at multiple levels have been reported. In 2/3 dogs and the single cat, a lymphocytic interface dermatitis was also described.

Immunopathology

In people as well as animals, PNP is known to be associated with production of autoantibodies targeting cadherins as well as plakins. Circulating antikeratinocyte IgG have been confirmed in 2/3 dogs and in the cat. When canine bladder was used as a substrate, serum IgG bound to the bladder epithelium in mostly an intercellular pattern, suggesting an antiplakin autoreactivity. Subsequently, in 2/3 dogs, serum samples precipitated several keratinocyte adhesion proteins such as DSG3, envoplakin and periplakin. A single canine PNP case also contained serum anti-DSC1 and anti-DSG1 IgG autoantibodies. In human PNP, autoantibodies against DSG3, DSG1, DSCs, envoplakin, periplakin, epiplakin, desmoplakin I and II, unnamed 170kDa protein and plektin have been uncovered.
References:


38. Werner LL, Brown KA, Halliwell RE. Diagnosis of autoimmune skin disease in the dog: Correlation between histopathologic, direct immunofluorescent and clinical findings. Veterinary immunology and immunopathology 1983; 5: 47-64.


43. Bizikova P, Dean GA, Hashimoto T, Olivry T. Cloning and establishment of canine desmocollin-1 as a major autoantigen in canine pemphigus foliaceus. Veterinary immunology and immunopathology 2012; 149: 197-207.


Mechanism of blister formation:
An intact skin is a critically important organ that functions as a first-line defense mechanism against physical and chemical damage. Its integrity is dependent on complex structures maintaining cell-cell and cell-matrix adhesion. Several autoimmune skin diseases disrupting this cohesion have been recognized. The mechanism by which this adhesion is disrupted varies depending on the type of disease.

a) Disruption of basement membrane adhesion – subepidermal blister formation due to dermo-epidermal separation (bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), epidermolysis bullosa acquisita (EBA), linear IgA disease (LAD), mixed autoimmune subepidermal blistering disease (mixed AISBD))

b) Disruption of keratinocyte adhesion – intraepidermal blister formation due to desmosome dissociation (pemphigus foliaceus (PF), pemphigus vegetans (PVeg), pemphigus vulgaris (PV), paraneoplastic pemphigus (PNP))

c) Keratinocyte injury and interface dermatitis – keratinocyte-targeting diseases causing disruption of epidermal cohesion (cutaneous lupus, erythema multiforme complex, etc.)

Cutaneous lupus erythematosus – an interface dermatitis with basal cell injury

Introduction
The first description of cutaneous lupus erythematosus (CLE) in dogs dates to 1979, when Griffin and colleagues reported a case of discoid lupus erythematosus (DLE).7 Since then, other forms of CLE have been recognized in dogs such as vesicular cutaneous lupus erythematosus (VCLE), exfoliative cutaneous lupus erythematosus (ECLE), mucocutaneous lupus erythematosus (MCLE), and generalized discoid lupus erythematosus (GDLE) (reviewed in 8). Using human classification, these lupus erythematosus specific skin diseases can be further divided into subacute LE (VCLE) and chronic LE (ECLE, MCLE, localized DLE and GDLE). An acute LE has not been described in dogs yet. Detailed review of CLE can be found in the comprehensive review (open access) by Banovic, et al.8

Subacute CLE

1. Vesicular cutaneous lupus erythematosus

Signalment
Although well-recognized, there is no information about the incidence and prevalence of this CLE form. The disease occurs equally between females and males, and collies and shelties are predisposed. The median age of onset is 5.5 (range:2-11 years).8

Clinical signs
Characteristic clinical signs include erythematous macules to minimally raised papules that rapidly progress to well-demarcated, deep erosions and ulcers, often of annular and/or polycyclic shapes. Most commonly affected areas are the glabrous skin of groin and axillae. Lesions can be found on concave pinnae, muzzle and, in rare cases, even in the oral cavity. Systemic signs are not typically seen in this disease; progression to systemic lupus erythematosus (SLE) has not been reported.

Treatment
Originally described cases of VCLE were successfully treated with immunosuppressive dosages of prednisone (2mg/kg/day) alone or in combination with azathioprine (2mg/kg/day). Treatment with pentoxifylline performed poorly. More recently, ciclosporin at median dosage of 5.5mg/kg/day was shown to be effective in most cases, while other required higher dosage or addition of topical tacrolimus or glucocorticoids. Majority of these cases received oral glucocorticoids during the initial treatment phase as well. Finally, a successful treatment of VCLE with mycophenolate mofetil has been recently published in a form of abstract.9

Subacute CLE

2. Exfoliative cutaneous lupus erythematosus

Signalment
The disease occurs equally between females and males, and collies and shelties are predisposed. The median age of onset is 5.5 (range:2-11 years).8

Clinical signs
Characteristic clinical signs include erythematous macules to minimally raised papules that rapidly progress to well-demarcated, deep erosions and ulcers, often of annular and/or polycyclic shapes. Most commonly affected areas are the glabrous skin of groin and axillae. Lesions can be found on concave pinnae, muzzle and, in rare cases, even in the oral cavity. Systemic signs are not typically seen in this disease; progression to systemic lupus erythematosus (SLE) has not been reported.

Treatment
Originally described cases of ECLE were successfully treated with immunosuppressive dosages of prednisone (2mg/kg/day) alone or in combination with azathioprine (2mg/kg/day). Treatment with pentoxifylline performed poorly. More recently, ciclosporin at median dosage of 5.5mg/kg/day was shown to be effective in most cases, while other required higher dosage or addition of topical tacrolimus or glucocorticoids. Majority of these cases received oral glucocorticoids during the initial treatment phase as well. Finally, a successful treatment of ECLE with mycophenolate mofetil has been recently published in a form of abstract.9
Chronic CLE

1. Localized (facial) discoid lupus erythematosus

Signalment
Facial DLE appears to be the most common form of CLE. The disease occurs more or less equally between females and males with males being slightly out-numbered. German shepherds and its crosses appear over-represented (about 1/3 of all reported cases). The median age of onset is 7 years (range: 1-12 years).

Clinical signs
Skin lesions of facial DLE consist of erythema, depigmentation, scaling, which may progress to erosions/ulcers and crusting, later followed by atrophy. Secondary infections are common. Lesions are usually present on the nasal planum, but may extend to the dorsal aspect of the muzzle (caudally to the nasal planum), lips and pinnae. Systemic signs are not typically seen in this disease; progression to systemic lupus erythematosus (SLE) has not been reported.

Treatment
Tetracycline antibiotics with or without niacinamide have been used in managing facial DLE for almost three decades. Because of the localized nature of this disease, topical treatment has been used as well. Tacrolimus applied twice daily for up to 10 weeks was safe and effective with significant clinical improvement seen in more than 70% of treated dogs.

2. Generalized discoid lupus erythematosus

Signalment
The current knowledge about this form of CLE is based on 10 cases described recently by Banovic and colleagues. The disease occurs equally between females and males. Breed predisposition has not been confirmed, but among the 10 dogs, two were Chinese crested dogs and two were Labrador retrievers. The median age of onset is 9 years (range: 5-12 years)

Clinical signs
Skin lesions of GDLE consist of multifocal to coalescing plaques with scaling, follicular casting and alopecia. Altered pigmentation can be appreciated with depigmentation and atrophy centrally on the plaque and hyperpigmentation at the periphery of the plaque. In some dogs, erosions and crusts may appear in the center of the plaque with atrophy and depigmentation. Variable degree of erythema can be also appreciated at the periphery of the plaques. Lesions affect trunk and neck predominantly. In some dogs (40%), plaques were visible near mucocutaneous junctions. Reticulated hyperpigmentation as seen in MCLE cases was present in two of the four dogs with lesions near mucocutaneous junctions. Systemic signs are not typically seen in this disease; progression to systemic lupus erythematosus (SLE) has not been reported in any of the 10 dogs from the largest case series. A single case report of an alopecic variant of chronic CLE progressing to SLE can be found in the literature.

Treatment
A variety of treatment regimens appears to be effective for managing GDLE including ciclosporin (with glucocorticoids during initial phase), hydroxychloroquine with topical tacrolimus and tetracycline/niacinamide combination.

3. Mucocutaneous lupus erythematosus

Signalment
The current knowledge about this form of CLE is based on total of 36. The disease affects females twice as more often as males. German shepherds and its crosses are over-represented (almost half of all dogs). The median age of onset is 6 years (range: 3-13 years).

Clinical signs
The typical skin lesions seen in MCLE are deep erosions and ulcer on mucocutaneous junctions, especially on the genitalia and anus, but also on the lips, eyelids and/or nasal planum. In contrast to mucous membrane pemphigoid, these erosive/ulcerative lesions do not cross into the mucosae. Scarring is also not a characteristic feature of MCLE. Most patients with perianal and perigenital lesions are painful, but systemic signs are not typically seen in this disease; progression to systemic lupus erythematosus (SLE) has not been reported.

Treatment
Oral prednisolone alone (1-2 mg/kg/day) or in combination with tetracycline antibiotic and niacinamide appear to reach complete disease remission the fastest. Complete remission can be also achieved with other immunosuppressive drugs such as ciclosporin (often combined with glucocorticoids during the initial phase). The efficacy of other immunosuppressants such as mycophenolate or azathioprine or other needs to be evaluated further. Topical creams/ointments (tacrolimus or glucocorticoids) can be considered as an adjunct treatment once the pain is resolved.
4. Exfoliative cutaneous lupus erythematosus

Signalment
Exfoliative CLE is a relatively rare form of chronic CLE that affects predominantly young German shorthaired pointers (GSP). In this breed, the mode of transmission is autosomal recessive. Similar disease might have been recognized in vizslas, which share a common ancestry with GSP (reviewed in 8). Females appear to be slightly over-represented (1.4x more than males). Dogs affected with this disease will usually start showing clinical signs during their first year of life (juvenile to young adult time of onset).

Clinical signs
The most commonly described skin lesions in ECLE include scaling and alopecia. Follicular casting and hyperpigmented patches to plaques with erythematous margin have also been observed. Erosions, ulcers and crusts, often affecting the muzzle and pinnae, were noted in advanced cases. Initial lesions are multifocal and patchy, but with time, they can acquire more generalized nature, mostly affecting trunk muzzle and pinnae. In contrast to other forms of CLE, many dogs with ECLE exhibit non-dermatological issues such as generalized lymphadenopathy, mild anemia, fluctuating thrombocytopenia and pain, which usually presents as a stiff gait and arched back. Interestingly, no joint abnormalities were found on examination (joint taps, X-rays) or post-mortem (personal experience and communication with Dr. Mauldin).

Treatment
Based on the cases reported in the literature, a treatment of ECLE represents a challenge and the majority of affected dogs will get eventually euthanized due to the lack of response. Variety of treatments with limited efficacy have been published including tetracycline and niacinamide, doxycycline, oral glucocorticoids, azathioprine, ciclosporin, leflunomide, hydroxychloroquine. In one study, hydroxychloroquine appeared to slow down the progression of the disease, while high-dose ciclosporin was not effective. A recent case managed by the author (PB) failed to respond to a combination treatment with ciclosporin (10 mg/kg/day), prednisolone (2 mg/kg/day) and hydroxychloroquine (10 mg/kg/day) after three months of treatment. A subsequent addition of mycophenolate mofetil (12 mg/kg twice daily) did not alleviate the ECLE signs during the additional eight weeks of treatment. The patient was euthanized because of the severity of skin lesions and perceived pain (hunched back, unwillingness to lay down on side), which was believed to be associated with the skin (joint exam, taps and radiographs were normal, while touching the skin triggered excessive skin twitching and signs of discomfort). Post-mortem did not reveal any joint abnormalities either.

Histopathology of CLE
Characteristic histological features of CLE are lichenoid cell-rich, lymphocytic interface dermatitis with basal keratinocyte vacuolar degeneration, apoptosis, loss of basal cells and basement membrane thickening. In facial DLE, these changes can be mild. Interface changes can involve the follicular infundibula, when lesions extend off of the nasal planum. In GDLE, the interface dermatitis is well developed and the hair follicle involvement is prominent. The hair follicle lesions consist of lymphocytic interface folliculitis involving infundibulum and extending to isthmus, lymphocytic mural folliculitis and sebaceous gland atrophy. In GDLE, rare apoptotic keratinocytes may appear in superficial epidermal layers. Histology of ECLE resembles that of GDLE, though epidermal orthokeratotic hyperkeratosis may be prominent in ECLE. The interface dermatitis with basal cell apoptosis in MCLE is often observed only in the close proximity to an ulcer. Some apoptosis can be observed off of the basal cell layer, but lymphocyte satellitosis, when present, was only mild in these upper epidermal layers. Like in facial DLE and GDLE, the interface dermatitis often extends to hair follicle infundibula and sometimes even to the isthmus. Concurrent bacterial infections are common. In VCLE, the interface dermatitis is characterized by prominent vacuolar degeneration and numerous basal keratinocyte apoptosis, which is sufficient to cause intrabasal clefting and epidermal sloughing. Hair follicle infundibula have similar lymphocytic and mural folliculitis. Occasional suprabasal apoptosis can be observed. Detailed review of CLE histopathology can be found in the comprehensive review (open access) by Banovic, et al.8
**Immunology of CLE**
The major immunological findings are summarized in the table below and additional details can be found in the comprehensive review (open access) by Banovic, et al.\(^8\)

<table>
<thead>
<tr>
<th></th>
<th>lupus band test</th>
<th>antinuclear antibodies</th>
<th>soluble nuclear antigen antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>vesicular CLE</td>
<td>yes (50% of cases)</td>
<td>no</td>
<td>yes (&gt;50%)</td>
</tr>
<tr>
<td>mucocutaneous LE</td>
<td>yes (almost all)</td>
<td>rare (low titer &lt;1:80)</td>
<td>not tested</td>
</tr>
<tr>
<td>facial DLE</td>
<td>yes (&gt;75% of cases)</td>
<td>?</td>
<td>not tested</td>
</tr>
<tr>
<td>generalized DLE</td>
<td>yes (90% of cases)</td>
<td>yes (low titer &lt;1:40)</td>
<td>not tested</td>
</tr>
<tr>
<td>exfoliative CLE</td>
<td>yes (100% of cases)</td>
<td>no</td>
<td>not tested</td>
</tr>
</tbody>
</table>

Table 1: Immunodiagnóstics and the findings in specific CLE forms
References:


4. Olivry T. An autoimmune subepidermal blistering skin disease in a dog? the odds are that it is not bullous pemphigoid. Veterinary dermatology 2014; 25: 316-8.


Autoimmune blistering skin diseases – basement membrane autoimmunity

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Mechanism of blister formation:
An intact skin is a critically important organ that functions as a first-line defense mechanism against physical and chemical damage. Its integrity is dependent on complex structures maintaining cell-cell and cell-matrix adhesion.\(^1\), \(^2\) Several autoimmune skin diseases disrupting this cohesion have been recognized. The mechanism by which this adhesion is disrupted varies depending on the type of disease.

a) Disruption of basement membrane adhesion – subepidermal blister formation due to dermo-epidermal separation (bullous pemphigoid (BP), mucous membrane pemphigoid (MMP), epidermolysis bullosa acquisita (EBA), linear IgA disease (LAD), mixed autoimmune subepidermal blistering disease (mixed AISBD))\(^3\), \(^4\)

b) Disruption of keratinocyte adhesion – intraepidermal blister formation due to desmosome dissociation (pemphigus foliaceus (PF), pemphigus vegetans (PVeG), pemphigus vulgaris (PV), paraneoplastic pemphigus (PNP))\(^5\), \(^6\)

c) Keratinocyte injury and interface dermatitis – keratinocyte-targeting diseases causing disruption of epidermal cohesion (cutaneous lupus, erythema multiforme complex, etc.)

Autoimmune subepidermal blistering diseases (AISBDs) – a basement membrane zone autoimmunity

Introduction
Spontaneously occurring autoimmune subepidermal blistering diseases (AISBDs) were described in dogs almost 40 years ago.\(^7\), \(^8\) The common pathomechanism shared by these diseases is the autoimmune response against structural proteins of the dermo-epidermal junction. Six diseases have been recognized in dogs based on the clinical phenotype and targeted antigen (Table 1).\(^4\) Some of these entities have been described in other animal species as well (Table 1). In contrast to people, the most common AISBD in dogs is mucous membrane pemphigoid (MMP; 48% of all AISBDs) followed by epidermolysis bullosa acquisita (EBA; 26% of all AISBDs). Bullous pemphigoid (BP), the most common AISBD in people is rarely seen in dogs (10% of all AISBDs),\(^4\) and other AISBDs are even rarer.
The mechanism of a blister formation is complex and, depending on the type of disease, the process involves antibodies (IgG and/or IgE and/or IgA), complement and/or various components of the immune system (neutrophils, eosinophils, mast cells, etc.). While the target autoantigens in AISBDs have been identified in dogs and, to some extent, in other species, little is known about the pathomechanism(s) of these diseases in domestic animals (antigen summarized in 4). Moreover, a commercially available array of antigen-specific immunoserological tests that could assist in distinguishing some of the diseases from each other does not exist in veterinary medicine. Therefore, the diagnosis of a particular AISBD in veterinary dermatology relies on a detailed clinical assessment and histopathology.

Detection of tissue-bound anti-BMZ antibodies or serum anti-BMZ antibodies, a test also not commercially available but fairly simple to perform, is unable to confirm the definitive diagnosis reliably, though it could inform us about the depth of the targeted antigen to some extent. Together with the clinical phenotype and histopathology, this information could further increase our confidence in the diagnosis made based on clinical features and histopathology. However, the diagnosis shall never be made based on a blood test only.4

This lecture will focus on the two most common canine AISBDs (MMP and EBA), the diagnostic dilemma AISBDs present for us, and using the knowledge about the disease pathomechanism, we will discuss the mechanism of action of some selected drugs used for treatment of these entities (note: the information about the disease pathomechanism is solely based on in vitro and in vivo research of the human counterparts).

1. Mucous membrane pemphigoid (MMP)

**Epidemiology and signalmment**

Mucous membrane pemphigoid is the most common AISBD recognized in dogs (48% of all AISBDs).4, 9 The disease occurs equally between females and males, and German shepherd dog and its crosses appear to be overrepresented (18/58 dogs; 31%).9, 10 The median age of onset is 6 years with most dogs developing MMP during their mid-adulthood (4-7 years; 50%) or at older age (≥ 8 years; 25%).9, 10

**Clinical signs**

The current knowledge about the clinical aspect of canine MMP is based on 50 cases.9, 10 Canine MMP affects primarily mucosa and mucocutaneous junctions in which the primary, though transient lesion is a vesicle and/or bulla. Rupture of these lesions leads to deep erosions and/or ulcers, found in most dogs (98%), often in a bilaterally symmetric pattern. Scarring is not reported frequently in dogs (16%), possibly due to the frequent oral cavity involvement (62%), which, like in people, is infrequently accompanied by obvious scarring signs.11 Other frequently affected areas in dogs include nasal planum (34%), ocular/periorcular area (20%), genital/perigenital area (16%) and concave ear pinnae (16%). About half of the reported cases exhibited systemic signs, like lethargy, as well as pain with eating, halitosis and hypersalivation. A loss-of-function of affected organs due to chronic scarring, as described in people, was not reported in dogs.10, 11

### Table 1: Autoimmune subepidermal blistering skin diseases in dogs

<table>
<thead>
<tr>
<th>Disease</th>
<th>Percentage of all AISBDs</th>
<th>Breed Predilection</th>
<th>Age Predilection</th>
<th>Characteristic Skin Lesions</th>
<th>Characteristic Lesion Distribution</th>
<th>Major Autoantigen</th>
<th>Minor Autoantigen</th>
<th>Histopathology</th>
<th>Other Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucous Membrane Pemphigoid</td>
<td>48</td>
<td>German shepherd</td>
<td>Middle-aged</td>
<td>tense vesicles (ear, deep erosions, ulcers, scarring, degeneration)</td>
<td>mucocutaneous junctions, mucosa</td>
<td>collagen XVII</td>
<td></td>
<td>Subepidermal vesiculation without or with minimal inflammation (autophagic and/or eosinophagic)</td>
<td>human, cat</td>
</tr>
<tr>
<td>Epidermolysis Bullosa Acropapulosa (EBA)</td>
<td>26</td>
<td>Great dane</td>
<td>Young</td>
<td>erythematous macules and papules; tense vesicles; deep erosions, ulcers</td>
<td>hemied skin (footpads, friction areas) and mucosa/mucocutaneous junctions</td>
<td>collagen VII</td>
<td>nd</td>
<td>Microscopic subepidermal vesiculation with variable degree of predominantly neutrophilic inflammation (interstitium eosinophils may be seen)</td>
<td>human, cat, pig (macaque)</td>
</tr>
<tr>
<td>Bullous Pemphigoid</td>
<td>33</td>
<td>nd</td>
<td>Middle-aged</td>
<td>erythematous macules and papules; tense vesicles; deep erosions, ulcers</td>
<td>hemied skin (footpads, friction areas) and mucosa/mucocutaneous junctions</td>
<td>collagen XVII</td>
<td>nd</td>
<td>Microscopic subepidermal vesiculation with variable degree of neutrophilic and/or eosinophagic inflammation</td>
<td>human</td>
</tr>
<tr>
<td>Junctional EBA</td>
<td>6</td>
<td>nd</td>
<td>nd</td>
<td>erythema, vesicles, deep erosions, ulcers and crusts</td>
<td>hemied skin (footpads, friction areas) and mucosa/mucocutaneous junctions (similar to DGB)</td>
<td>lemniscus-552</td>
<td>nd</td>
<td>Microscopic subepidermal vesiculation without inflammation or with variable neutrophilic and/or eosinophagic inflammation</td>
<td>human, cat, pig (macaque)</td>
</tr>
<tr>
<td>Mixed AISBD</td>
<td>4</td>
<td>nd</td>
<td>nd</td>
<td>erythema, vesicles, deep erosions, ulcers and crusts</td>
<td>hemied skin (footpads, friction areas) and mucosa/mucocutaneous junctions (similar to BP)</td>
<td>collagen XVII</td>
<td>nd</td>
<td>Microscopic subepidermal vesiculation with mixed neutrophilic and eosinophagic inflammation</td>
<td>human, cat, pig (macaque)</td>
</tr>
<tr>
<td>Linear IgA Disease (LAD)</td>
<td>1</td>
<td>nd</td>
<td>nd</td>
<td>erythematous papule, erosions, ulcers and crusts</td>
<td>mucosa (oral cavity) and hemied skin (ears, nasal planum, extremities)</td>
<td>collagen XVII (secreted)</td>
<td>nd</td>
<td>Microscopic subepidermal vesiculation without or with minimal neutrophilic inflammation</td>
<td>human, cat, pig (macaque)</td>
</tr>
<tr>
<td>Bullous systemic Lupus Erythematosus (SLE)</td>
<td>1</td>
<td>nd</td>
<td>nd</td>
<td>erythema, vesicles, deep erosions, ulcers and crusts</td>
<td>hemied skin, footpads and mucosa/mucocutaneous junctions</td>
<td>collagen VII</td>
<td>nd</td>
<td>Microscopic subepidermal vesiculation without inflammation or with variable, predominantly neutrophilic inflammation</td>
<td>human, cat, pig (macaque)</td>
</tr>
</tbody>
</table>

* Reference 4; nd - not determined (due to insufficient number of reported cases); major autoantigen - antigen recognized by more than 50% of affected individuals.
Histopathology
Histopathological features of canine MMP are similar to that reported in people. Subepidermal or submucosal vesicles, if not ruptured, were usually devoid of inflammation. Dermal and submucosal inflammation is variable and mild to moderate amount of both neutrophils (73%) and eosinophils (55%) can be seen below the vesicles or intact epithelium. Rowing of individual neutrophils and/or histiocytes along the basement membrane was less common than that seen in canine epidermolysis bullosa acquisita.

Immunopathology
Most dogs affected with MMP possessed tissue-bound autoantibodies, predominantly IgG (92%), and complement C3 (63%) deposited along the basement membrane zone. Circulating autoantibodies, predominantly IgG and less frequently IgE, could be detected using salt-split canine buccal mucosa tissue in 71% and 32% of dogs, respectively. Like dogs, people affected with MMP, especially those with lesions confined to the oral cavity, do not always possess detectable anti-basement membrane autoantibodies. Similarly to people, canine MMP has been shown to be immunologically heterogeneous with autoantibodies targeting proteins of the basement membrane such as collagen XVII, BP230 or laminin-332. Autoactivity against other basement membrane proteins such as α6β4 integrin, laminin-311 or collagen VII, as seen in some affected people, has not been confirmed in dogs yet.

Summary
Canine MMP is a naturally occurring chronic and recurrent AISBD that preferentially affects mucosae and mucocutaneous junctions. Although a fairly rare disease, MMP is the most common AISBD in dogs and it represents a disease homologue to its human counterpart.

Other animal species:
A naturally occurring MMP has been described in two cats (one of the two cats from an older publication on bullous pemphigoid (case #1) fits clinically, histopathologically and immunologically for MMP). Both cats exhibited vesicles and/or erosions and ulcers on mucosae and mucocutaneous junctions (eyelids (1), lips (2), soft palate (1)), and concave pinnae (2). Histopathology revealed dermo-epidermal separation with none to minimal dermal inflammation composed of dendritic/histiocytic cells and occasional neutrophils and eosinophils. Immunotesting revealed autoantibodies targeting collagen XVII in one and laminin-332 in another cat.

Pathomechanism of blister formation in human MMP
The pathomechanism of canine MMP has not been studied; however, considering the clinical, histological and immunological similarity with its human counterpart, similar mechanism(s) could be involved. The development of MMP in people, like the other AISBDs, has been associated with particular HLA haplotypes. The loss of immunological tolerance results in production of IgG antibodies of variable specificity. Multiple basement membrane proteins have been shown to act as autoantigens, including collagen XVII (BP180; NC16A or C-terminus), laminin-332, the 120kDa ectodomain of BP180, laminin-311, integrins, etc.) (reviewed in ). From these, collagen XVII (PB180) and laminin-332 are considered to be the major autoantigens in people with MMP. While the collagen XVII is also the major target autoantigen in BP, the major pathogenic epitopes in people are different between PB and MMP.

The pathomechanism(s) leading to blister formation in MMP is not fully understood. The pathogenicity of anti-laminin-332 IgG has been confirmed by a passive transfer of purified anti-laminin-332 IgG from people with MMP into a mouse with a human skin graft. The blister formation was complement-independent as a passive transfer of anti-laminin-332 Fab fragment resulted in a blister development. The most recent study showed that, at least in some people with MMP, an internalization of collagen XVII is observed in their skin biopsies and that anti-collagen XVII IgG from some MMP-affected people triggered collagen XVII internalization in the exposed keratinocyte culture. A pathomechanism similar to that described in BP, a macropinocytosis, is suspected to be involved in this process. Considering the immunological heterogeneity of human MMP, further studies are needed to elucidate other pathomechanisms involved in the blister formation in this disease.

2. Bullous pemphigoid (BP)

Epidemiology and signalement
In contrast to people, BP is rarely seen in dogs (10% of all AISBDs). While older reports of canine BP can be found in the veterinary literature, many of these cases would be today given a different diagnosis such as MMP, EBA or vesicular cutaneous lupus erythematosus after applying the current clinicopathological criteria for these diseases. The first well-characterized canine BP case dates to mid 1990s. Because of the rarity of this disease and the small number of reported cases, a statement about breed- or sex-predilection cannot be made. The disease usually starts, like many other autoimmune diseases, in the middle adulthood (median age of onset: 5 years).
Clinical signs
The only available case series of seven dogs provided insight about the clinical picture of canine BP. In these dogs, skin lesions consisted of erythematous macules, patches or plaques (4/7; 57%), tense vesicles or bullae (3/7 dogs; 43%) erosions or ulcers (6/7; 86%), as well as crusts (5/7; 71%). Lesions were present on the back (4/7; 57%), pinnae (4/7; 57%), axillae (2/7; 29%), and abdomen (2/7; 29%). Footpads were rarely affected in dogs with BP (1/7; 14%), a contrasting finding to that observed in dogs with EBA. Lesions at mucosal or mucocutaneous junction sites were detected in four dogs (57%) and involved predominantly the oral cavity (3/7; 43%) and lip margins (4/7; 57%).

Histopathology
Like in other AISBDs, dermo-epidermal separation leading to vesicle formation was a typical finding in BP. Formed vesicles contained variable numbers of neutrophils (71%) and eosinophils (67%). Similar inflammatory infiltrate was detected in the upper dermis. In some dog, numerous degranulated mast cells were depicted in the upper dermis.

Immunopathology
Three (75%) and two (50%) of the four tested dogs with BP possessed tissue-bound IgG and IgM autoantibodies, respectively, deposited along the basement membrane zone. Tissue-bound complement (C3) was seen in one of the four tested dogs (25%). Circulating autoantibodies, predominantly IgG, could be detected using salt-split canine buccal mucosa tissue in 100% of dogs with BP. Similarly to people, sera from dogs with BP contain autoantibodies targeting the NC16A domain of collagen XVII.

Summary
Canine BP is a naturally occurring AISBD with collagen XVII autoreactivity affecting predominantly haired skin. Mucosal and mucocutaneous junction involvement is seen in about 50% of dogs with BP and footpad sloughing, in contrast to EBA, is only a rare feature. This disease is a clinical, histopathological and immunological homologue of the human BP.

Other animal species:
A naturally occurring BP has been described in cats13, pigs31, horses32 and, possibly, in a rhesus macaque. In cats, lesions of BP appear to be of minimal severity, with vesiculation and erosions occurring predominantly on the ears, trunk and extremities. Mucosal involvement can be seen, but appears to be mild. Like in people and dogs, the BP affected cat produced IgG against NC16A domain of collagen XVII. In horses with BP, vesicles appeared suddenly and progressed rapidly into erosions and ulcers covered with crusts. The lesions were widespread with especially prominent oral ulceration. Systemic signs such as lethargy and anorexia accompanied the skin lesions. Euthanasia was elected for humane reasons due to the severity of their disease. Sera from horses with BP contained IgG against the NC16A domain of collagen XVII.

Pathomechanism of blister formation in human BP
Several pathomechanisms involved in the blister formation in BP through IgG (complement-dependent, complement-independent) or IgE autoimmunity have been proposed.

i) IgG and complement-dependent pathway:
Historical observations of IgG and C3 tissue deposits at the basement membrane zone of affected people, presence of circulating anti-collagen XVII IgG in the serum of affected people, and the results from IgG-dependent animal models led to the proposal of a complement-dependent pathway in which anti-collagen XVII IgG binding to the basement membrane zone is followed by a complement activation, mast cell degranulation and recruitment of neutrophils, which, through their granule content, degrade collagen XVII and create a blister.

ii) IgG (IgE) and complement-independent pathway:
However, histologically, some BP patients will show blisters devoid of any inflammation with only a scant inflammatory infiltrate in the underlying dermis. Moreover, IgG4, an antibody unable to activate complement, is often detected in BP patients and, interestingly, this autoantibody can induce dermo-epidermal separation. Complement-independent pathway, therefore, has been proposed (reviewed in). In this pathway, anti-collagen XVII IgG (also IgE; see below) will, upon binding, lead to internalization of the protein by macrophagocytosis and subsequent loss of adhesion. Also, it has been shown that binding of these autoantibodies will stimulate keratinocytes to produce IL-6 and IL-8, which leads to recruitment of neutrophils without the help of complement, and to FcεR-independent activation of mast cells.
iii) IgE and its role in bullous pemphigoid:
In people, pruritic urticarial erythema, eosinophilic infiltration of the affected skin, elevated IgE (>50% of people), circulating anti-collagen XVII IgE (>70% of people), and eosinophilia (50% of people) have been observed. These observations suggested the role of IgE and Th2 polarization in the pathogenesis of BP. Interestingly, the majority of the anti-collagen XVII IgE is bound not to the basement membrane zone, but to the mast cell surface in the superficial dermis. Similarly, IgE autoantibodies could bind to the FcεRI detected on eosinophils and basophils in BP-affected individuals. A possible mechanism leading to activation and degranulation of mast cells and eosinophils could be through a direct binding of cleaved collagen XVII ectodomains to the collagen XVII-specific IgE bound to dermal mast cells and eosinophils (reviewed in 35). Other mechanisms could involve stimulation of IL-6 and IL-8 or macropinocytosis of collagen XVII upon binding to the collagen XVII in the basement membrane.

3. Epidermolysis bullosa acquisita (EBA)
Epidemiology and signalment
Epidermolysis bullosa acquisita is the second most common AISBD in dogs (26% of all AISBDs). Most affected dogs were young (median: 1.2 years) males (male:female = 2.3:1) with lesions developing before one year of age in almost half of them (45%). Interestingly, while childhood EBA has been recognized in people, this disease affects mostly people in the fourth to fifth decade of their lives. In the largest case series of canine EBA, great danes were overrepresented (55%).

Clinical signs
The current knowledge about the clinical aspect of canine EBA is based on 20 cases. Few other cases reports can be found in the literature. Like in other AISBDs, characteristic skin lesions seen in dogs with EBA were tense vesicles and bullae (90%) progressing with time into deep erosions and ulcers (100%). Additional lesions included erythematous macules and patches (75%) or papules and wheals (40%). Lesions were usually detected in the oral cavity (95%), concave pinnae (80%) and glabrous skin of groin and axillae (50% and 75%, respectively). In contrast to dogs with MMP and BP, dogs with EBA often exhibited footpad sloughing (70%). Pruritus and pain have been reported in 38% and 85% of affected dogs, respectively, and systemic signs such as fever, lethargy, lymphadenopathy and anorexia were seen in most cases (94%).

Histopathology
Detailed histopathological description can be found for 17 dogs with EBA. Like in other AISBDs, dermo-epidermal separation leading to vesicle formation was a typical finding in EBA. Formed vesicles were devoid of any inflammation (76%) or contained variable numbers of neutrophils (94%). In some dogs, eosinophils were seen intermixed with neutrophils (41%). In early lesions, small subepidermal vacuoles as well as rowing of neutrophils and/or histiocytes were seen along the basement membrane zone. Superficial, dermal perivascular to interstitial inflammation composed of neutrophils (100%) was frequently seen. A low number of eosinophils intermixed among neutrophils was seen in 71% of dogs.

Immunopathology
Most dogs affected with EBA possessed tissue-bound autoantibodies, predominantly IgG (81%), deposited along the basement membrane zone. Circulating autoantibodies, predominantly IgG and less frequently IgE, could be detected using salt-split canine buccal mucosa tissue in 90% and 21% of dogs, respectively. Similarly to people, sera from dogs with EBA contained autoantibodies targeting the NC1 domain of collagen VII.

Summary
Canine EBA is a naturally occurring AISBD with collagen VII autoreactivity affecting mucosae as well as haired skin, especially friction and pressure areas. This disease is a clinical, histopathological and immunological homologue of the human EBA.

Other animal species:
A naturally occurring EBA has not been reported in any other animal species.
Pathomechanism of blister formation in human EBA
Investigation of human patients and animal models has contributed to our knowledge about pathogenesis of EBA. As with other autoimmune diseases, a loss of tolerance is considered to be the initial step in the establishment of autoimmunity. Indeed, human and experimental EBA are strongly associated with certain MHC class II alleles.\textsuperscript{48, 49} In general, the pathogenesis of EBA can be subdivided into i) induction phase (antibody production), ii) antibody maintenance phase and iii) effector phase (antibody binding, effector cell recruitment, blister formation).\textsuperscript{49} Findings from the most recent animal models suggest that classic antigen-presenting cells (especially B cells, dendritic cells and macrophages), CD4 T cells, especially those with Th1-like cytokine profile, as well as GM-CSF and neutrophils are key cellular and molecular requirements for antibody production and clinical disease development.\textsuperscript{49-52} Interestingly, special neutrophils localized in the marginal zone of a spleen have been shown to play an important role in, so called, T cell-independent antibody production by B cells.\textsuperscript{51} These neutrophils called B cell helper neutrophils ($N_{BH}$) secrete B cell-stimulating factors such as BAFF, APRIL, IL-21, and their crosstalk with B cells enhances generation of ready-to-use antibodies against already known antigen. Indeed, depletion of neutrophils in immunization-induced EBA mouse model resulted in a lower autoantibody production.\textsuperscript{52} Anti-collagen VII antibodies (IgG) then bind to basement membrane, which leads to subsequent complement activation, release of anaphylatoxins (i.e. C5a) and recruitment of neutrophils.\textsuperscript{49} Additionally, GM-CSF, CXCL1/2, IL-1$\alpha$/$\beta$ and LTB$_4$ produced from recruited neutrophils and other, yet-to-be-determined, cells contributes to the perpetuation of the neutrophil recruitment.\textsuperscript{49, 52-53} Neutrophils activated through Fc$\gamma$R (specifically the Fc$\gamma$RIV based on the recent murine model) then secrete reactive oxygen species (ROS) and matrix metaloproteinases (MMPs), which leads to direct damage of the basement membrane zone and blister formation.\textsuperscript{49, 49} Mast cells, which play important role in BP, do not appear to be involved in the EBA pathogenesis.\textsuperscript{54} The pathomechanism of the noninflammatory EBA is currently unknown.
References:


4. Olivry T. An autoimmune subepidermal blistering skin disease in a dog? The odds are that it is not bullous pemphigoid. Veterinary dermatology 2014; 25: 316-8.


In veterinary medicine, systemic immunosuppression is the cornerstone of management of numerous immunological disorders. Using our knowledge about the immunopathomechanism, autoimmune and immune-mediated diseases in veterinary dermatology could be separated into two main groups: i) autoantibody-mediated diseases, and ii) lymphocyte-mediated diseases (Figure 1). Understanding the pathomechanism of these diseases and understanding the mechanism of action of drugs used for their treatment is important and allows us to: i) select an appropriate drug or combination of drugs to treat the disease of interest, ii) predict the speed of clinical effect, iii) make us aware of potential side-effects, and iv) determine how to best monitor for those side effects (Figure 1).

This lecture will review selected immunosuppressants and their mechanism of action. Their possible effects will be explained using selected immune-mediated and autoimmune skin diseases such as pemphigus, epidermolysis bullosa acquisita and cutaneous lupus erythematosus.

**Target 1: Lymphocytes**

**Glucocorticoids**

Glucocorticoids (GCs) affect many important functions throughout the body such as glucose, protein and fat metabolisms, water excretion, calcium balance, response to stress, and alter the function of multiple organ systems including cardiovascular, renal, muscular-skeletal, nervous and immune systems. These countless functions are exerted through the glucocorticoid receptor (GR), a member of the nuclear receptor family of transcription factors. In the absence of ligands, GRs reside in the cytoplasm, inactive in complexes with chaperon molecules. Upon ligand binding by GCs, GRs can act directly in the cytoplasm by interfering with signal transducers involved in T cell receptor signaling (JNK, PI3K), or, more typically, ligand-bound GRs translocate into the nucleus where GRs act on transcription of several pro- and anti-inflammatory genes. The effects can be: i) direct activation by binding to the glucocorticoid response elements (GRE) within the regulatory regions of target genes, ii) direct repression by binding to negative GRE (nGRE) or iii) trans-repression by binding of GRs to another transcription factor (e.g. NF-κB, STAT, AP-1). In addition, GCs exert non-genomic effects on cells, which include direct effects on cell membranes, adhesion molecules, membrane ion channels, etc. Indeed, there is evidence that high doses of systemic GCs can control acantholysis (keratinocyte dissociation seen in pemphigus) by means other than immunosuppressive effects. This involves direct effect on keratinocytes, especially on the strength of keratinocyte adhesion molecules and their phosphorylation (e.g. an increased transcription of desmosomal cadherins). Such effects could explain the rapid response of lesions in pemphigus-affected individuals, especially when receiving a high-dose GC pulse therapy. Because of the diversity of mechanisms by which GCs affect variety of cells inside and outside of the immune system, their effects extend from lymphocytes though autoantibody production and up to a local tissue inflammation. Not surprisingly, they are one of the most commonly used drugs in numerous autoimmune and immune-mediated skin diseases.
**Ciclosporin**

Ciclosporin (CsA) belongs to a group of immunosuppressants called calcineurin inhibitors. Although originally used to prevent allograft rejection and later licensed for treatment of canine and feline atopic dermatitis, CsA has been used in other immune-mediated dermatoses of dogs and cats including cutaneous lupus, erythema multiforme, pemphigus foliaceus, sebaceous adenitis, vasculitis, perianal fistulae, urticaria pigmentosa, etc.\(^6\) The main mechanism of action involves inhibition of the calcineurin/nuclear factor of activated T cells (NFAT) pathway (reviewed in\(^7,8\)). NFAT is well known transcription factor that, upon activation of T cells, binds the IL-2 gene promoter and activates production of this cytokine. By inhibiting the NFAT signaling pathway in T cells, CsA impairs the expression of IL-2 and negatively affects subsequent T cell proliferation. Therefore, CsA is indicated in numerous lymphocyte-mediated autoimmune and immune-mediated diseases. As the production of autoantibodies depends on T cell – B cell interactions, CsA will affect autoantibody-mediated diseases as well, though a delay in the onset of the effect should be expected in this group of diseases.

The NFAT family consists of five members, four of which require calcineurin for their activation (NFAT1–4). Although the NFAT pathway in T cells has been studied extensively for more than two decades, its role in other immune cells has only recently been uncovered. Indeed, at least one NFAT family member is expressed by almost every cell type in the body, and its signaling contributes to the hematopoiesis as well as innate and adaptive immune functions. Table 1 summarizes some of the many effects of NFAT inhibition in cells of the immune system.\(^7,9\)

This “multiple-hit” effect involving the inhibition of T cell activation, proliferation and differentiation, as well as altered function and cytokine expression by myeloid cells (macrophages, dendritic cells, neutrophils, mast cells, eosinophils) could explain the high efficacy of CsA in management of different immune disorders. Most of the effects on cells other than lymphocytes are, however, seen only at higher CsA dosages. Indeed, opportunistic infections, especially fungal or viral, are rarely seen in patients receiving CsA as a monotherapy and at lower dosages (<10 mg/kg/day), but may be observed in patients on higher CsA dosages and/or when combined with other immunosuppressants.\(^10\) The susceptibility to opportunistic fungal infections could be due to the altered function of neutrophils and their ability to deal with fungi.\(^11\)

### Table 1: Demonstrated impact of NFAT inhibition on cells of the immune system (reference: \(^9\))

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>NFAT Function</th>
<th>Effect of NFAT Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells</td>
<td>• Cytokine expression: IL-2, -3, -4, IFN(<em>\gamma), -10, -12, TNF(</em>\alpha), GM-CSF, &lt;br&gt;• Activation, differentiation and proliferation of T cells &lt;br&gt;• Treg function, expansion, and survival</td>
<td>• Altered activity of T cells in many aspects&lt;br&gt;• Altered help to other immune cells (e.g. B cells, macrophages, etc)</td>
</tr>
<tr>
<td>dendritic cells</td>
<td>• Cytokine expression: IL-2, -12, -10 &lt;br&gt;• Activation of NK cells</td>
<td>• Decreased T and NK cells activation</td>
</tr>
<tr>
<td>Mast cells</td>
<td>• Cytokine expression: IL-2, -4, -13, -31, TNF(_\alpha) &lt;br&gt;• Enhanced survival after Fc(\gamma)RI activation</td>
<td>• Disruption of pro-Th2 priming&lt;br&gt;• Anti-itch/inflammation/allergy</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>• Degranulation, cytokine release (GM-CSF, IL-2, -4)</td>
<td>• Impaired degranulation and cytokine release</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>• Regulation of production and expression of prostaglandins &lt;br&gt;• Anti-fungal properties</td>
<td>• Increased susceptibility to fungi</td>
</tr>
<tr>
<td>Macrophages</td>
<td>• Cytokine expression: IL-6, -10, -12, -23, TNF(_\alpha) &lt;br&gt;• Regulation of TLR-induced genes (e.g. iNOS expression)</td>
<td>• Decreased TLR response&lt;br&gt;• Decreased inflammation</td>
</tr>
<tr>
<td>NK cells</td>
<td>• Cytokine expression: TNF(<em>\alpha), IFN(</em>\gamma), GM-CSF &lt;br&gt;• Proliferation and cytotoxicity &lt;br&gt;• Expression of adhesion molecules and migration</td>
<td>• Altered cytotoxicity and tissue infiltration&lt;br&gt;• Altered expression of proinflammatory cytokines</td>
</tr>
</tbody>
</table>

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Oclacitinib
Janus kinases (JAKs) are intracellular, non-receptor tyrosine kinases that are involved in signaling of more than 60 different cytokines and cytokine-like hormones (e.g. variety of interleukins, interferons, colony-stimulating factors, prolactin, erythropoietin, growth hormone, etc). There are four members of this family, JAK1, JAK2, JAK3 and TYK2. Cytokines are critical for regulation of immune responses involved in the host defense, and are also key players in exaggerated and misdirected immune responses seen in autoimmune diseases. Because of the central function of JAKs in cytokine signaling and immunity, JAKs have become a new target for development of immunosuppressive drugs. The expected impact on cytokines upon inhibition of a particular JAK is listed in Table 2.

Oclacitinib is the first selective JAK inhibitor approved by the FDA in dogs. Specifically, it has been approved for management of itch and inflammation in dogs with allergic skin diseases. Although listed as a selective JAK1 inhibitor, like the other JAK inhibitors used in people, the inhibitory effect of oclacitinib extends beyond the JAK1 (the potency rank is reported to be JAK1>JAK2>TYK2>JAK3). Importantly, JAK2 and TYK2, which are important in hematopoiesis and Th1-type response, respectively, appear to be inhibited by oclacitinib only weakly, as suggested by the high half maximal inhibitory concentration (IC50) levels obtained in the cell-based assays (>1000 nM and >3000 nM for receptor containing combination JAK2/JAK2 and JAK2/TYK2, respectively). In contrast, the half maximal inhibitory concentration (IC50) levels for receptors containing JAK1 ranged between 36 to 249 nM. Because of the preferential effect on Type I/II cytokines, many of which are known players in inflammatory and autoimmune conditions, oclacitinib presents a novel drug with a high potential. Indeed, the use of oclacitinib in diseases other than allergic have been discussed among veterinary dermatologists, and anecdotal reports of its successful use have been published recently. In people, other JAK inhibitors, for example tofacitinib (JAK3/JAK1) or baricitinib (JAK1>JAK2), are being used or tested for diseases such as rheumatoid arthritis, psoriasis, vitiligo or alopecia areata.

Table 2: Expected impact of JAK inhibition on specific cytokines and hormones. (references: http://www.cellsignal.com/reference/pathway/jakstat_utilization.html)

<table>
<thead>
<tr>
<th>JAK</th>
<th>Affected molecules</th>
<th>Effect of inhibition</th>
<th>Predicted side effects (general)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK1</td>
<td>γc family: IL-2, 4, 7, 9, 15</td>
<td>Impaired T cell proliferation and differentiation</td>
<td>Infected lipemia/hypercholesterolemia</td>
</tr>
<tr>
<td></td>
<td>gp130 family: IL-6, 11</td>
<td>Impaired signaling by a wide range of cytokines; especially Th2 type</td>
<td>Possible impairment of NK cell functions</td>
</tr>
<tr>
<td></td>
<td>IL-31</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IFNα/β, IFNγ, IL-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TSLP</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EGF, PDGF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JAK2</td>
<td>EPO, TPO, IFNγ</td>
<td>Impaired erythropoiesis and myelopoiesis</td>
<td>Infections</td>
</tr>
<tr>
<td></td>
<td>gp130 family: IL-6, 11</td>
<td></td>
<td>Anemia</td>
</tr>
<tr>
<td></td>
<td>/pc family: IL-3, 5, G- and GM-CSF</td>
<td></td>
<td>Neutropenia</td>
</tr>
<tr>
<td></td>
<td>IL-12 / IL-23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-31</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GH, prolactin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JAK3</td>
<td>γc family: IL-2, 4, 7, 9, 15</td>
<td>Impaired T cell proliferation</td>
<td>Possible NK cell lymphopenia (not observed yet)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Defective signaling through γc cytokines</td>
<td>Infections</td>
</tr>
<tr>
<td>TYK2</td>
<td>IFNα/β</td>
<td>Impaired Th1 and Th17 response</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL12 / IL-23</td>
<td></td>
<td>Infections</td>
</tr>
</tbody>
</table>

Abbreviations: TSLP thymic stromal lymphopoietin, EGF epidermal growth factor; PDGF platelet-derived growth factor, EPO erythropoietin, TPO thrombopoietin, GH growth hormone, G-CSF granulocyte-colony stimulating factor, GM-CSF granulocyte, macrophage-colony stimulating factor
Other lymphocyte-targeting drugs

Azathioprine

Azathioprine is one of the most commonly used immunosuppressive drugs in veterinary dermatology in situations when GCs fail to induce disease remission or for maintenance therapy to avoid a long-term use of GCs at higher dosages. It is an antimetabolite pro-drug that transforms in the body into 6-mercaptopurine, which then blocks purine metabolism and interferes with DNA synthesis. Lymphocytes, cells dependent on de novo purine synthesis, are most sensitive to its effect. Indeed, azathioprine has been shown to reduce T and B cell proliferation in dogs after a short-term use (2mg/kg daily for 7 days).\(^\text{16}\) Interestingly, a myelosuppression is a fairly uncommon side effect in dogs and, in contrast to people, it does not appear to correlate with a low activity of one of the enzymes involved in the metabolism (thiopurine methyltransferase (TPMT)). Indeed, the activity of TPMT in dogs is generally much higher than that reported in people.\(^\text{17, 18}\) In people, a higher activity of TPMT, an enzyme that metabolizes 6-mercaptopurine into 6-methyl mercaptopurine (6-MMP), has been linked to a higher risk of hepatotoxicity.\(^\text{19}\) It is therefore possible that the higher TPMT activity demonstrated in dogs could be linked to the hepatotoxicity observed in some dogs receiving azathioprine.\(^\text{20, 21}\)

In contrast, cats and horses have very low activity of TPMT.\(^\text{22}\) Therefore, cats are very sensitive to this drug and myelosuppression has been reported with markedly lower dosages than those given to dogs.\(^\text{23}\) The risk of myelosuppression in horses at standard dosages is lower due to the low bioavailability of azathioprine in this species.\(^\text{24}\)

Considering the pharmacokinetics of this drug and its metabolism, in dogs, the recommended initial dosage of azathioprine is 2 mg/kg once daily. In case of lack of response after one month and in the absence of side-effects, the dose can be escalated up to 3 mg/kg/day in 0.5 mg/kg increments. A careful monitoring for liver toxicity is recommended (every 2 weeks during the first 3 months; look for rapid rises in ALT activity and total bilirubin, but keep in mind that the GCs given concurrently will also increase ALP and, to some degree, ALT activities).

In cats, azathioprine should be given at a much lower dosage (possibly 0.5 to 1.0 mg/kg every 2 days), and cats must be monitored for the development of myelosuppression.

Little is known about azathioprine in horses. The most recent publication recommended a dosage of 3 mg/kg/day.\(^\text{25}\) Monitoring for the development of myelosuppression is warranted.

Chlorambucil

Chlorambucil, a nitrogen mustard alkylating agent, alkylates and cross-links DNA during all phases of the cell cycle, resulting in disruption of DNA function, cell cycle arrest and apoptosis. As such it has effect on T and B cells and has been used for treatment of several autoimmune skin diseases. In small animals, it is usually given at dosages 0.1-0.2 mg/kg daily to every other day. Monitoring for the development of myelosuppression is warranted.

Mycophenolate mofetil

Mycophenolate mofetil (MMF) is a prodrug of mycophenolic acid (MPA), an inhibitor of inosine-5'-monophosphate dehydrogenase. MPA depletes guanosine nucleotides preferentially in activated T and B lymphocytes and inhibits their proliferation, thereby suppressing cell-mediated immune responses and subsequently an antibody formation. Mycophenolate is being used with variable success in immune-mediated and autoimmune skin diseases at dosages usually ranging from 10 to 15 mg/kg twice daily, though higher dosages or three times daily administration have been reported in the literature.\(^\text{26, 27}\) Higher dosages or three times daily administration are believed to increase the risk of gastrointestinal issues (diarrhoea, haematochezia, vomiting). Except for the gastrointestinal side-effects, MMF appears to be overall safe and frequent blood test monitoring for myelosuppression appears to be not necessary in most patients.

Target 2: Antibodies

Intravenous immunoglobulins (IVIg)

Intravenous immunoglobulin (IVIg) are liquid or freeze-dried, sterile therapeutic preparations containing human G isotype immunoglobulins (Ig) made from a pool of plasma of healthy human blood donors. They have been used for variety of autoimmune and immune-mediated skin diseases at dosages between 1 to 3 g/kg. Although IVIg possess myriad of immuno-modulatory effects, removal of pathogenic IgG is currently proposed to be one of the main mechanisms involved in the relatively rapid improvement in human patients with pemphigus or other antibody-mediated diseases.\(^\text{28}\)
This function is delivered through the interaction between IVIg and the neonatal Fc receptor (FcRn). The FcRn protects IgG from degradation, thereby allowing for the long half-life of this class of antibody in the serum. The Fc portion of IgG binds with high affinity to FcRn, which is primarily expressed in intracellular organelles of the endothelial cells. Under normal circumstances, circulating IgG are taken up by fluid phase pinocytosis by endothelial cells, where they bind to FcRn. This allows for sorting and transportation of the IgG back to the cell surface, where it is released back to the circulation. In contrast, IgG that do not bind to FcRn after uptake into cells enter lysosomal compartments and undergo degradation. Blocking the FcRn by high dose of IgG through the IVIg infusion will enhance the degradation of the pathogenic antibodies. Indeed, IVIg treatment in people with pemphigs reduced the level of pathogenic IgG by more than 50% during the first week after the administration. This supports the current opinion that IVIg increase the catabolism of IgG. Further, confirmation that FcRn plays a major role in the effect of IVIg in pemphigus was demonstrated by Li and colleagues, who showed that IVIg injection to a wild-type experimental mouse will reduce the levels of pathogenic IgG and prevent blister formation, while IVIg injection to an FcRn-deficient mouse will not show any benefit. There is only limited evidence of efficacy of IVIg in dogs with antibody-mediated skin diseases. Up to today, only two cases (one PF and one EBA) have been identified in English-written literature. Although rapid improvement of clinical signs appears to be present in both cases, a cautious interpretation is merited until larger case series or ideally controlled studies are available. Because of the broad spectrum of effects, IVIg have also been used for lymphocyte-mediated skin diseases. The use of IVIg is very safe, but one must bear in mind that the use of IVIg in dogs carries the same potential adverse effects like in people (hyper-coagulation, renal failure, hypotension) in addition to the potential hypersensitivity or anaphylactic reaction due to the introduction of xenoproteins.

**Target 3: Tissue inflammation**

**Doxycycline and Niacinamide**

A combination of these two drugs has been used for management of autoimmune and immune-mediated skin diseases for many decades. Both drugs have a good safety margin and a variety of anti-inflammatory properties. For example, doxycycline has been shown to disrupt inflammatory cytokine pathways (IL-1, IL-6, IL-8, TNF, etc.), inhibit function of matrix metalloproteinases, reduce leukocyte chemotaxis and function of nitric oxide synthase. Niacinamide, a vitamin B3, is an endogenous inhibitor of poly(adenosine diphosphate-ADP)-ribose polymerase 1 (PARP-1). Niacinamide inhibits multiple pro-inflammatory cytokines, neutrophil chemotaxis via reduced ICAM-1 expression and B cell differentiation and other. The safety margin of these drugs is high, and, as a combination, they have been used in several autoimmune/immune-mediated skin disorders (pemphigus, CLE, AISBDs, etc).

**Colchicine**

Colchicine is a natural product that can be extracted from two plants of the lily family. The main mechanisms are inhibition of neutrophil chemotaxis, adhesion and mobilization, and superoxide production in addition to inhibition of NACHT-LRRPYD-containing protein 3 (NALP3) inflammasomes involved in interleukin (IL)1β processing and release. The anti-neutrophilic effect of colchicine is exerted by its ability to bind to tubulin, the monomeric component of microtubules. Binding of the tubulin prevents the tubulin polymerization into microtubules, which are important for neutrophil motility. Colchicine therefore affects the elasticity (stiffness) and viscosity of neutrophils and, thereby, regulates cell motility and the ability of neutrophils to migrate through small tissue channels into the site of action. Because of this action, colchicine has been tried anecdotally in neutrophilic dermatoses such as epidermolysis bullosa acquisita or sterile pustular neutrophilic dermatitis of miniature schnauzers. The most common side effects are gastrointestinal upset (nausea, vomiting, diarrhea, abdominal pain). As colchicine is a p-glycoprotein substrate, the concurrent use of cyclosporine (another immunosuppressant with a potential to manage this condition) is not recommended.

**Hydroxychloroquine**

Hydroxychloroquine (HCQ) is an antimalarial agent with anti-inflammatory properties and it is currently one of the drugs of choice in severe or generalized forms of cutaneous lupus erythematosus (CLE) in people (an excellent treatment algorithm can be found in the following review paper). The efficacy of HCQ in treatment of CLE could be explained by a variety of immunomodulatory effects such as inhibition of TLR7 and TLR9 signaling on pDC with a subsequent disruption of the production of inflammatory cytokines (IFNs, TNFα, IL-6), inhibition of prostaglandin production, changes in antigen presentation, etc.

In veterinary medicine, HCQ has been used successfully for treatment of exfoliative CLE and generalized discoid LE. In all four dogs treated with HCQ, the agent appeared to be effective to either induce full remission (one case of generalized DLE) or to stop the disease progression (2/3 dogs with exfoliative CLE). The duration of the treatment in the three positively responding cases ranged from 7 to 12 months and the dosage was 5 mg/kg (one dog) or 10 mg/kg (two dogs) once daily. In two dogs receiving the higher dosage, a regular echocardiogram, ophthalmologic examination and blood work did not reveal any abnormalities. The dog treated with the 5 mg/kg dosage exhibited mild retinal degeneration at one year recheck, but this was concluded to be age-related most likely.
Summary
Autoimmune and immune-mediated skin disease are complex entities in which lymphocytes, (antibodies) as well as local tissue inflammation play a role. A successful treatment is able to manage all aspects of the inflammation either with a single agent targeting the key step in the pathogenesis, or with a drug with a broad spectrum of effects acting on multiple aspects of the pathogenesis.
Proposed treatments combinations based on the known disease pathomechanism and/or previously published data:

1. pemphigus variants, systemic lupus erythematosus, vasculitis, VKH
   a. start with an oral glucocorticoid (preferably with a more effective pulse protocol?)
   b. if no response, as described above, add azathioprine (dogs) or CSA (dogs and cats) or chlorambucil (cats) or mycophenolate (dogs)

2. pemphigoid variants:
   a. start with an oral glucocorticoid
   b. add tetracycline or doxycycline + niacinamide
   c. if no response after 2 months, add immunosuppressants as for pemphigus

3. cutaneous lupus variants:
   see algorithm (Figure 2)

4. vitiligo and alopecia areata:
   a. start topical tacrolimus or cyclosporine or a JAKinib (human data)

5. sebaceous adenitis:
   a. cyclosporine

Figure 2: Treatment logarithm for management of CLE
References:


Transdermal flux studies of a new antimicrobial containing lasalocid for methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) infections of the skin.

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Background – Topical antimicrobial preparations are critical treating suspected and confirmed MRSP infections due to the increasing incidence of widespread resistance to systemic antibiotics. Lasalocid has been shown to be efficacious against MRSP. Topical formulations containing this antimicrobial may become very important in the treatment of canine pyoderma due to MRSP, if they are demonstrated to be concentrated well in the skin with minimal systemic penetration.

Hypothesis/Objectives – To determine the effect of various formulation types on the penetration and retention of lasalocid applied to canine skin in vitro.

Methods – An in vitro, open study to assess the effect different formulations types have on transdermal penetration and retention of lasalocid in canine skin. Transdermal penetration of each formulation was assessed throughout a 24 h period and retention of lasalocid was assessed at the conclusion of the 24 h period.

Results – Retention of the active ingredient was greatest for the solution (292.75 ± 141.67 µg/cm²), followed by the lotion (143.93 ± 96.38 µg/cm²), then the ointment (39.46 ± 12.31 µg/cm²). The solution method had significantly higher skin retention and proportion of applied dose retained in skin than the lotion and ointment methods (Tukey test, p<0.01).

Conclusions and clinical importance – The active ingredient lasalocid was not identified in the receptor fluid in any sample indicating that systemic absorption of the active ingredient in vivo is unlikely. The solution resulted in the greatest skin retention and may be the most useful formulation going forward. Lasalocid may be useful in treatment of MRSP infections in future.

Sources of funding:
Australian and New Zealand College of Veterinary Scientists
Luoda Pharma

Conflicts of interest:
One of the authors, Stephen Page, works for Luoda Pharma, one of the sources of funding for this study.
The term “adverse food reactions (AFRs)” encompasses immunological reactions as well as other reactions such as food intolerance or poisoning. The clinical signs can be cutaneous or extra-cutaneous. Cutaneous AFRs (CAFRs) in small animals are almost exclusively of immunological nature and, therefore, the term cutaneous AFRs is often used interchangeably with food allergies. Cutaneous AFRs in small animals are believed to be predominantly IgE-mediated. In some food allergic dogs, a lymphocyte-activation by food allergens has also been demonstrated, though this latter pathomechanism has not been studied in depth.

This lecture will focus on CAFRs in dogs and cats, and it will discuss several selected topics:

i. How common are CAFRs in dogs and cats?

ii. Are food-induced atopic dermatitis (dog) and pruritus (cat) clinically different from those triggered by airborne allergens?

iii. How long should elimination diet trial last?

iv. What are the common food allergens?

v. Why do I prefer to use extensively hydrolysed diets for elimination diet trials?

These questions will be answered during the lecture using recently published critically appraised topics on food allergy (open-access publications).

How common are CAFRs in dogs and cats?

Twenty-eight publications contained usable information about the prevalence of CAFRs in dogs and cats.² The prevalence of CAFRs among dogs and cats presented to veterinarians for any health issue is fairly low (1-2% in dogs and <1% in cats). The range of prevalence of CAFRs was markedly higher among dogs presented to the veterinarian for pruritus (9-40%), for any allergic skin disease (8-62%) or for atopic dermatitis (9-50%). In cats, the range of prevalence of CAFRs was fairly similar between cats with pruritus (12-21%) and cats with any allergic skin disease (5-13%).

Altogether, in dogs and cats with pruritus or confirmed allergic skin disease, the prevalence of CAFRs is high enough to warrant an elimination diet trial to address the role of food in their skin issues.

Are food-induced atopic dermatitis (dog) and pruritus (cat) clinically different from those triggered by airborne allergens?

Several publications published over the years compared food-induced atopic dermatitis to that triggered by airborne allergens in dogs. Based on the largest study (843 dogs), food-induced atopic dermatitis is clinically similar to that triggered by airborne allergens. Some features, however, may be more common in food-induced atopic dermatitis. These include strictly non-seasonal character, very early or late age of onset (<1 year or >6 years), concurrent gastrointestinal signs, less frequent involvement of eyelids and lower responsiveness to glucocorticoid treatment. Interestingly, features such as perianal and perioral pruritus were shown to be not exclusive for food-induced atopic dermatitis or CAFRs in general, and were often more common in dogs with flea-bite hypersensitivity and atopic dermatitis triggered by airborne allergens.

The pattern of pruritus in cats in relationship to the allergenic trigger was evaluated in 502 cats. The proportion of distribution of skin lesions was similar between the different allergenic triggers (food, flea, airborne), though the food-induced pruritus appeared to involve head and neck slightly more frequently.

In summary, the clinical differences between food-induced atopic dermatitis and pruritus and those triggered by other allergens are subtle and often very minor to be used to reliably distinguish one trigger from another. Therefore, the diagnosis of CAFR is based on exclusion of the other triggers, a positive response to an elimination diet trial and subsequent flare up upon a food challenge.
How long should elimination diet trial last?
Variable lengths of elimination diet trial ranging from three to 13 weeks can be found in the published literature. Based on the data analysis from a recent publication, eight publications addressed this question sufficiently. These eight studies included 209 dogs and 40 cats. Based on the information from the 209 dogs with CAFR, it was estimated that, after 3 weeks of a diet change, approximately half of dogs achieved a marked reduction of their signs. From 5 weeks onward, signs had returned to normal in more than 85% of dogs, and this percentage increased to over 95% if extending the dietary trial to 8 weeks. Fewer than 5% of dogs needed the elimination diet for up to 13 weeks to reach complete remission of signs of CAFR. Cats needed approximately 4, 6 and 8 weeks of a restriction diet trial to achieve remission of clinical signs in 50, 80 and 90% of cats, respectively. Altogether, to diagnose CAFR in at least 80% of dogs and cats, a restriction diet trial should last at least 5 weeks in dogs and 6 weeks in cats. Increasing the duration of the diet trial to 8 weeks will increase the sensitivity to more than 90% in both species. Nonetheless, the duration of the elimination diet trial should be flexible and should depend on the patient’s response (i.e., food challenges can be started shortly after the patient’s disease becomes well controlled). When predicting the duration of the elimination diet trial, one should consider the severity and chronicity of the skin lesions and the presence of complicating factors.

What are the common food allergens?
A literature search to address this question yielded 16 publications in which the offending food allergen was identified based on a positive food challenge. From the 297 dogs, 34% of dogs were allergic to beef, 17% to dairy products, 15% to chicken, 13% to wheat, 6% to soy and 5% to lamb. Some of the less common allergens included corn (4%), egg (4%), pork (2%), fish and rice (2%). Barley, rabbit, chocolate, kidney bean and tomato were also reported; each as an individual single case. Information about food allergens was found in 78 cats and positivity to beef (18%), fish (17%), chicken (5%), wheat, corn and dairy products (each 4%) and lamb (3%) was recorded. Egg, barley and rabbit, each a single cat, were also reported as offending allergens.

Although the historically published studies identified these most common allergens, one should bear in mind that some allergens might have gotten under-reported due to the limited number of challenges performed in the studies. And while it is prudent to avoid the most common allergens when selecting the patient’s elimination diet, the ultimate choice of elimination diet and subsequent challenge diets should be based on the patient’s dietary history.

Why do I prefer to use extensively hydrolysed diets for elimination diet trials?
Selection of a diet for an elimination diet trial has become more and more challenging. Indeed, due to the broad spectrum of different protein sources available over-the-counter, the identification of truly novel protein diet became almost impossible in some cases. Moreover, recent studies on food allergen cross-reactivity in humans as well as some of the early serological data in veterinary medicine suggest that patients sensitized to one food may react to another food sharing the same or similar allergen (e.g., beef and lamb or chicken and fish or corn and rice and potatoes). Finally, a recently published critically appraised topic demonstrated high prevalence of mislabeling among novel/limited ingredient diets. In this study, 18 original studied and one abstract were identified to contain information about the rate of diets containing undeclared proteins using various methods including PCR, ELISA, microscopic evaluation or mass spectrometry. These various methods uncovered ingredients not listed on the label in 0-83% of tested diets (median: 45%); this percentage varied between 33 and 83% in diets labeled as novel/limited ingredient diets advertised for elimination diet trials. Among the evaluated diets, there were several hydrolysed diets. Mislabeling or missing ingredients was found in one of the hydrolysed diet; although it is not clear if the mislabeling was due to a contamination during processing or due to a possibility that glutelin type B1 with high sequence similarity to that of the rice is also present in potatoes.

A variety of hydrolysed diets for dogs and cats can be found on the market. The degree of hydrolysis affects their performance as a diagnostic diet. Indeed, between 20-50% of dogs allergic to the native protein exhibited worsening of their allergies when fed partially hydrolysed diets. Extensive protein hydrolysis is required to dampen the IgE-mediated as well as clinical recognition in dogs allergic to the native allergen. In summary, with the increasing knowledge about possible cross-reactivity between evolutionary related and unrelated species and contamination of novel/limited ingredient diets with other proteins, the use of these diets for diagnostic purpose has become less attractive. Extensive protein hydrolysis has been shown to abolish serological and clinical recognition by animals allergic to the native protein and, therefore, an extensively hydrolysed diet represents a valuable diagnostic tool for dogs and cats with CAFRs. Due to the high risk of clinical reactivity to the partially hydrolysed diets (20-50%), these diets are likely best to be used as a diagnostic tool in animals suspected not be allergic to the native protein.
References:
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Food allergies in humans: risk factors and preventative strategies.

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Introduction:

Food allergy is an adverse response to the usually harmless food that is mediated by the immune system and characterised by production of IgE antibodies specific to the trigger food. The majority of food allergies in humans are triggered by 8 common foods, peanut, tree nuts, cow’s milk, hen’s egg, fish and shellfish, soy and wheat. The prevalence of IgE-mediated food allergy in children is reported to have increased over recent decades, and it is now recognised as a significant public health burden in many developed countries. Australia reports the highest prevalence of IgE-mediated food allergy internationally with 10% of infants challenge-confirmed allergic to one or more foods. In other developed countries prevalence estimates range from 1 to 5%. Hospital admissions for food-induced anaphylaxis in Australian children aged 0-4 years have increased 5-fold between 1994-1995 and 2004-2005, and the most recent data indicates that this is yet to have plateaued, with food-induced anaphylaxis admission rates continuing to rise until 2012. Significant advances have been made in understanding the causes of food allergy in children. The development of food allergy is multifactorial in origin and these multiple contributing factors have recently been summarised as the “5D’s”: dry skin & diet, dogs & dirt, and vitamin D which pertain to three separate hypotheses.

Hypothesis 1: Dry Skin and Diet - The Dual Allergen Exposure Hypothesis

Eczema and food allergy commonly co-occur. Around 1 if 5 infants with eczema in the first year of life will go on to develop a food allergy, and the risk is higher for early-onset and more severe eczema requiring topical corticosteroids. The role of dry skin and diet in the development of food allergy is described by the ‘dual-allergen exposure hypothesis’ first proposed by Lack in 2008. This hypothesis proposed that in infants with disrupted skin barrier function, as occurs in eczema, sensitisation occurs via low-dose allergen exposure through the skin. Oral tolerance, the active suppression of and immune response against food antigens, is subsequently induced through timely exposure to allergens via the oral route. However if oral tolerance fails to occur then food allergy can develop.

There is also a growing body of evidence from observational studies and randomised controlled trials (RCTs) which demonstrate that the early introduction of allergenic foods into the infant’s diet is associated with a reduced risk of developing food allergy. This supports that early oral exposure is an important step for inducing oral tolerance. Therefore, preventative strategies against food allergy development are aimed at the early introduction of allergenic foods into the infant’s diet and infant feeding guidelines both in Australia and internationally have been updated to reflect this. RCTs are currently in progress which aim to assess whether regular application of moisturisers in infancy are effective in prevention eczema, and whether this has a follow-on effect in reducing the risk of food sensitisation and allergy.

Hypothesis 2: Dogs and Dirt - The Hygiene Hypothesis

The hygiene hypothesis proposes that a lack of exposure to infections and microbes in early childhood modulates the development of the immune system and predisposes children to developing allergic disease. This hypothesis was first proposed in 1989 with the observation that allergies were more common in children who had fewer older siblings. The authors proposed that greater exposure to infections in childhood by older siblings bringing more infections into the home, may be protective against developing hay fever. This hypothesis is now supported by a growing body of evidence. Children who are raised on farms and have frequent contact with animals are less likely to develop allergic disease compared to children raised in cities. Children with older siblings, who attend childcare in early life or are exposed to pet dogs are also less likely to develop food allergies. One study also reported that cleaning of infants pacifiers by parents sucking on it, as opposed to rinsing it in tap or boiling water, was associated with a reduced risk of developing childhood allergic diseases. In this study, the infant’s oral microbiota also differed between the two cleaning methods which suggests that the development of allergy was related to immune system modulation from microbes transferred from the parent’s saliva to the infant.
These factors, and others, have been shown to impact the composition of the gut microbiota, which plays an important role in the development of the immune system in early life. Differences in intestinal microbiota composition have been demonstrated between allergic and non-allergic children and recent studies suggest that the risk of food allergy is associated with changes in the gut microbial composition. Following on from this concept, a number of studies have investigated the role of probiotics in allergy prevention, however a recent meta-analysis found little evidence to support that probiotic supplementation reduced the risk of food allergy.

**Hypothesis 3: Vitamin D**

The role of vitamin D in the development of food allergy was first hypothesised on the basis of two lines of enquiry. Firstly, a strong latitude and food allergy association has been reported in several studies. Countries further from the equator with lower ambient ultraviolet radiation, a proxy for vitamin D levels, have reported higher rates of food allergy, adrenaline-autoinjector prescriptions and hospital admissions for food-related anaphylaxis. Secondly, season of birth, another proxy for vitamin D levels, has been associated with an increased risk of food allergy and adrenaline-autoinjector prescriptions.

The first evidence of directly measured vitamin D and the risk of food allergy has only recently been demonstrated. In the HealthNuts study, serum vitamin D levels were measured in 577 infants who underwent oral food challenges. Infants with vitamin D insufficiency (<50 nmol/L) were 11-times more likely to have peanut allergy (adjusted OR, 11.51; 95% CI, 2.01-65.79) and nearly 4-times more likely to have egg allergy (adjusted OR 3.79; 95% CI, 1.19-12.08) than those with normal vitamin D levels. Further exploration of this cohort also showed that genetic polymorphisms associated with lower vitamin D-binding protein levels which increase the biological availability of serum vitamin D, reduced the association between low vitamin D levels and food allergy.

Paradoxically, both vitamin D deficiency and excess have been implicated in the development of food allergy and there is evidence to support a “U-shaped” association where both high and low vitamin D levels in cord blood in the same population demonstrated an increased risk of allergic outcomes. Due to the ambiguity of these results, well-designed RCTs are needed to understand the potential of vitamin D supplementation as a preventative intervention for food allergy. The VITALITY study is double-blind, randomised, placebo-controlled trial currently recruiting infants in Melbourne, Australia, to investigate whether the use of vitamin D supplementation in infancy is effective in preventing food allergy. Results are eagerly anticipated.

**Summary**

Current research continues to advance our understanding of the causes of food allergy. The strongest evidence for preventative strategies comes from randomised controlled trials on early peanut introduction which has resulted in changes to infant feeding guidelines internationally. Beyond this, few preventive strategies have been implemented at the population level. Results from trials on other allergenic solids, vitamin D supplementation, and eczema prevention are eagerly anticipated in order to inform future preventative strategies.
References


Introduction:

Food allergy is adverse response to a usually harmless food that is mediated by the immune system. Symptoms of IgE-mediated food allergy occur shortly after exposure to the food and include urticaria, angioedema, vomiting and anaphylaxis, a severe and sometimes life-threatening allergic reaction. There are currently no curative treatments for food allergy available in routine practice in Australia. Management of food allergy primarily involves allergen avoidance and administration of medical treatment following accidental exposure, such as antihistamines and adrenaline-autoinjectors. Despite vigilant efforts by most, accidental exposures may still occur and families can experience significant anxiety around dietary choices and fear of severe reactions. Therefore, quality of life is significantly impacted for children with food allergies, and their families.

The natural history of food allergy:

Food allergy results from either a failure to establish oral tolerance or the breakdown of existing oral tolerance. Oral tolerance is the active inhibition of immune responses to food proteins previously encountered by the gastrointestinal tract. A proportion of children with food allergy will naturally outgrow the disorder indicating that oral tolerance can subsequently be achieved in previously allergic individuals. Approximately 80% of children allergic to egg and cow’s milk will develop tolerance in early childhood, in contrast to peanut and tree nut allergy where only 20% of children are expected to outgrow their allergies with most persisting into adolescence and beyond. Children who have outgrown food allergy have higher circulating regulatory T cells compared to children who remain allergic and oral tolerance may occur from the suppression of allergic immune responses by these regulatory T cells. An increase in circulating IgG4 cells, which compete with IgE for binding sites to mast cells, has also been observed in children who develop tolerance.

Food allergy phenotypes and the natural history of food allergy:

Different phenotypes of food allergy may have an impact on the natural history and likelihood of developing tolerance. Approximately 70-80% children who are allergic to egg in its raw or lightly-cooked form are able to tolerate egg in baked foods, such as cakes and biscuits. Egg allergy may therefore be distinguished into two phenotypes on the basis of baked egg reactivity, that is, “raw and baked egg allergic” versus “raw egg allergic, baked egg tolerant”. The protein epitopes in egg can be sequential or linear, comprising of several amino acids in a row, or conformational (coiled). Extensive heating of egg induces changes in the conformational structure of the epitope, effectively destroying it, and thus reducing its allergenicity. Children who produce IgE antibodies that predominately recognise conformational epitopes can therefore consume baked egg without reaction. In addition, heating egg protein with wheat flour forms a food matrix, which also reduces the allergenicity of the protein thereby affecting the digestibility of the proteins or making the IgE binding sites less accessible.

In observational studies, children who were “raw egg allergic, baked egg tolerant” were more likely to outgrow their egg allergy compared to those who were “raw and baked egg allergic”. In addition, children who ate baked egg frequently were more likely to outgrow their egg allergy compared to those who ate it infrequently. Similar observations have been reported for baked milk, with most milk allergic children able to tolerate milk and baked foods. Extensive boiling of peanuts reduces the allergenicity of peanut with some proteins leeching into the cooking water.

Oral immunotherapy and other treatments for food allergy

New treatments for food allergy aim to induce immunomodulatory responses which suppress the allergic response to re-exposed food antigens with the ultimate goal of inducing oral tolerance. Oral immunotherapy (OIT) as a treatment for food allergy has been the subject of much research over the last decade. It involves feeding increasing doses of the food allergen on a daily basis. Protocols and doses vary considerably between foods and studies. OIT generally involves
3 phases, initial dose escalation, gradual dose build-up and the maintenance phase. Initial dose escalation usually occurs in a single day and involves ingesting extremely small doses up until 10-25mg of the food is consumed without reaction. The purpose of this phase is to find a safe starting point for the amount of food that can be ingested at home without reaction. This is followed by the build-up phase where the dose increases incrementally every 1-2 weeks, depending on the protocol. Both the initial dose escalation and build-up phase need to occur in a clinical setting in case of an allergic reaction. The build-up phase continues until the patient is able to tolerate the maximum dose on the protocol, which may be 500-4000mg. This phase can take 3-9 months. Once the maintenance does is reached, daily consumption of the maintenance dose continues for 6 to 18 months.25, 26

Several outcomes are described in OIT trials which are important to distinguish. Desensitisation refers to an increase in the reaction threshold of the food allergen that causes an allergic reaction while still remaining on active treatment. Desensitisation may protect against accidental exposure to the allergen, but only while active therapy continues. Sustained unresponsiveness is the absence of clinical reaction to the food allergen that persists after treatment has ceased for a certain period of time.26

Overall, clinical trials show that OIT is effective in inducing desensitisation to peanut, egg and cow’s milk.24, 26-28 A recent meta-analysis which included data from 27 trials and 1171 participants found that there was a substantial beneficial effect of allergen immunotherapy in achieving desensitisation (RR 0.16, 95% CI0.10-0.26). This estimate combined results from trials which assessed different foods (egg, peanut and cow’s milk) and also different forms of allergen immunotherapy (oral and sublingual). For the outcome of sustained unresponsiveness, that is, a persistent lack of clinical reaction after ceasing OIT, evidence of a beneficial effect was suggested but not statistically significant, (RR 0.29 95% CI 0.08-1.13).27 A recently updated Cochrane review has assessed the evidence for egg allergen immunotherapy. The authors pooled results from 10 randomised controlled trials which included 439 participants and found that children receiving OIT were more likely to be able to tolerate a full serving of egg after OIT compared to children in the control group (RR 4.25, 95% CI 2.77-6.53). However they also found that children receiving OIT experienced frequent adverse events, with 75% experiencing mild to severe allergic reactions and 8% experiencing adverse reactions which required adrenaline.28

The body of evidence supports that OIT is effective in inducing desensitisation and may be able to induce sustained unresponsiveness.27 Fewer studies have assessed whether sustained unresponsiveness can be achieved after cessation of therapy and it has only be observed in some of the individuals who achieved desensitisation. In addition, it is unclear whether continued allergen exposure is required to maintain sustained unresponsiveness, and if so, how much and how often. Standardisation of OIT protocols are also lacking and researchers have not yet determined the optimal induction and maintenance scheduling or whether this differs by patient age or underlying severity of disease. It must be also noted that allergic reactions occur frequently during OIT, including anaphylaxis and new cases of eosinophilic esophagitis have been reported in several children undergoing OIT.25, 28

A recent area of interest is whether the addition of an adjuvant, such as probiotics or dietary starches, enhance the tolerogenic response and improve the outcomes of OIT.29 In a randomised, double-blinded placebo controlled trial, 74% of children undergoing peanut OIT with adjuvant probiotic achieved sustained unresponsiveness and were able to tolerate peanut 2-6 weeks after ceasing OIT, compared to 3% in the placebo group. In a follow-up study, 67% of these children were continuing to eat peanut 4 years after the initial OIT study finished.29, 30 Other studies have trialled using modified food allergens, such as baked egg and boiled peanut.31, 32 Different routes of administration for immunotherapy have been trialled in a small number of studies including sublingual immunotherapy and epicutaneous immunotherapy. These therapies show some efficacy in inducing desensitisation although less so than OIT, but have better safety profiles.25, 26 A small number of other therapies have been researched including peanut vaccines and herbal formulas although limited data is available.33, 34
References


Agreement and correlation between intradermal allergy testing and IgE serology performed at three different dermatology specialist centres

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**Aims**
The aim of this study was to compare the results of intradermal allergy testing and an IgE serological test (Heska Allercept) to determine the agreement and correlation between the two on an individual allergen basis.

**Methods**
Cases of canine atopic dermatitis were recruited at three dermatology referral centres in Australia and subjected to both tests. 119 dogs were included in the study, with 33 from Site 1, 36 from Site 2 and 50 from Site 3.

To eliminate regional variation in the content of intradermal testing kits, only those allergens that were included at all three test sites and were also present in the IgE serological test were included in the analysis. 43 allergens were common to all tests. For each allergen, there were four possible outcomes: a positive result in both tests (designated $I^+H^+$); a negative result in both tests ($I^-H^-$); a positive skin test but a negative Heska test ($I^+H^-$); or a negative skin test and a positive Heska test ($I^-H^+$). Neither test was considered to be the “Gold standard” against which the other was compared. Instead, the overall agreement between the tests was calculated by summing the number of double positive and double negative results for each allergen. Accordingly, discordant results were calculated by summing the results in which one of the tests was positive when the other was negative. For the purposes of analysis, a good level of agreement was arbitrarily chosen to be 85% or higher. In other words, 85% agreement would indicate that for a particular allergen in all patients tested, the two tests were in agreement 85% of the time. To further analyse the relationship between the skin test results and the IgE serology, for each allergen, the strength of the skin test result (0 to 4) was correlated with the Heska score (EA units) using Spearman’s rank correlation coefficient.

**Results**
The overall agreement (average of all allergens) between the two tests at the Sites 1 and 2 was 84% and 88% respectively, compared to 72% at Site 3.

At Site 1, the tests agreed at least 85% of the time for 22 of the 43 allergens. For 10 of these 22 allergens, this high level of agreement was attributable to both tests being negative. For example, for Alternaria, the tests agreed 100% of the time, but this is because the results in both tests were negative in all animals tested. Other allergens that showed high agreement due to being negative in both tests were ant, cockroach, Cypress, Elm, Daisy, Mugwort, and the remaining three moulds. For 12 of the 22 allergens, the 85% or greater agreement occurred due to the combination of double positive and double negative results. These were Dermatophagoides farinae, Birch, Black Willow, Eucalyptus, Liquidambar, Maple, Plane, Privet, Timothy, Dandelion, Perennial ragweed and Sheep Sorrel.

At Site 2, the tests agreed at least 85% of the time for 30 of the 43 allergens. For 19 of these 30 allergens, this high level of agreement was attributable to both tests being negative. For the remaining 11 of the 30 allergens, the 85% or greater agreement occurred due to the combination of double positive and double negative results. These allergens were Dermatophagoides pteronyssinus, Maple, Brome, Kentucky Blue, Phalaris, Sweet Vernal, Timothy, Daisy, Fat Hen, Mugwort and Sheep Sorrel. The tests that showed high agreement at both the Sites 1 and 2 were Maple, Timothy and Sheep Sorrel. At Site 3, only two allergens resulted in agreement in at least 85% of the tests performed (Cypress and Yellow Dock).

Disagreement between the tests occurred when a positive result occurred in one with a negative result in the other. At Site 1, the skin test was more likely to be positive than IgE serology for the three environmental mites. The IgE serology was more likely to be positive for flea, and a number of tree, grass and weed pollens.

At Site 2, positive skin tests in the absence of positive IgE serology were seen at least 10% of the time for ant, cockroach, Dandelion and Red Root Amaranth. Positive IgE serology in the absence of a positive skin test was seen over 10% of the time for Tyrophagus putrescentiae, flea, Yellow Dock and a number of tree and grass pollens.

The major difference in the results obtained at Site 3 was the very high rate of skin test reactivity in the absence of positive IgE serology. Apart from six allergens, every other allergen yielded a positive skin test reaction in the absence of positive IgE serology in at least 10% of patients. For many allergens, the figure was much higher than this, peaking at 34% for flea. In other words, in 34% of the 50 patients tested, there was a positive skin test result
for flea in the absence of positive IgE serology (the highest percentage of positive skin tests in the absence of positive IgE serology at either of the other two sites was 19% for cockroach at Site 2).

Correlation between intradermal allergy test results and IgE serological scores varied widely from allergen to allergen. Site 1 had the highest number of significant correlations (19 allergens) compared to 4 at Site 2 and 10 at Site 3. Allergens which yielded significant correlations at two sites were *Dermatophagoides farinae*, *Tyrophagus putresceantiae*, Maple, Melaleuca, Sweet Vernal, Timothy, Fat Hen, Perennial Ragweed, Sheep Sorrel and Yellow Dock.

**Conclusions**

The results of this study suggest that variations in intradermal allergy testing technique and interpretation may explain the different levels of agreement between skin testing and IgE serology. This provides further evidence that reliance on the intradermal test as a “Gold Standard” is erroneous.
Mechanisms of action of allergen specific immunotherapy

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Historical view of the action of immunotherapy

The development of allergen specific immunotherapy was founded in early studies that aimed to elucidate the cause of hay fever. In 1819, John Bostock first differentiated seasonal hay fever from other forms of catarrh (such as bronchitis) and in 1873 Blackley advanced evidence in favour of pollen being the cause. In 1903 Dunbar reported that the skin and mucus membranes of hay fever sufferers were sensitive to a “toxin” from pollen that didn’t cause a reaction in normal individuals. When this toxin was injected into animals, it resulted in the formation of an antitoxin that could neutralise this toxin and prevent allergen induced conjunctival inflammation. Dunbar’s antiserum was marketed as Pollantin and administered by nasal, intraocular, bronchial inhalation and subcutaneous injection. Pollantin was derived from immunised horses and rabbits, whereas a competing product, Graminol was derived from herbivores who were thought to be naturally resistant to pollen toxin because they were constantly eating pollinating grasses. Pollantin was known to provide some relief to hay fever sufferers, but it didn’t effect a permanent cure and the results were hit and miss.1

The use of Pollantin in Britain resulted in Dunbar, and his junior associate, Carl Prausnitz, coming across Britain’s foremost vaccinologist, Sir Almroth Wright, Director of the Inoculation Department at London’s St Mary’s Hospital. In his research institute, further exploration of active immunisation against pollen toxin was explored, but this was abandoned when Prausnitz injected Dunbar with a pollen extract and he had a violent systemic reaction.2

However, working in Wright’s Institute were two physician scientists, Leonard Noon and John Freeman, and these followed up the abandoned experiments on active immunisation using pollen extracts. Noon interpreted Dunbar’s data as being the result of antibodies, and summarised that curing hay fever relied on the induction of active immunity. He hypothesised that repeated exposure to the toxin at short intervals was more likely to induce hypersensitivity. In 1911, Noon published the first short term clinical trial of inoculation using sub-cutaneously injected pollen extracts from Timothy grass and showed that the sensitivity of patients could be dramatically reduced.1 Noon’s work was continued by John Freeman who reported the clinical outcomes in 20 patients treated with pollen extracts. Freeman noted that the immunity to the pollen toxin appeared to be due to production of antibodies.3

Blocking antibodies

The subsequent discovery of “reagin”, a heat labile antibody thought to be responsible for positive skin tests in allergic patients (now known to be IgE), and a heat stable antibody that was induced by immunotherapy (now known to be IgG), led to the theory of blocking antibodies as the primary mechanism of action of immunotherapy. The concept of blocking antibodies was first described by Cooke in 1935, who was the first to demonstrate that allergic serum mediated skin reactions could be blocked by incubating the allergen extract with heat stable, nonreaginic antibodies.4 Loveless subsequently demonstrated that the heat stable, blocking antibodies were binding to the same antigen as the reaginic sensitising antibody.5

The role of blocking antibodies in the mechanism of action of immunotherapy has been vigorously debated with some investigators believing them to be highly relevant whereas others considering them insignificant. Despite there being no doubt that immunotherapy induces the production of IgG antibodies, in particularly of the IgG4 subclass, the lack of correlation between the concentration of allergen specific IgG blocking antibodies and clinical outcomes meant that the blocking antibody theory fell out of favour. For example, Djurup11 statistically analysed a number of studies that had evaluated IgG4 levels and concluded that a high IgG4 concentration at the end of treatment was associated with a failure to respond. Ewan10 reported that IgG levels did not correlate with clinical outcome of bee venom immunotherapy.

However, other studies have regarded this correlation as too simplistic to explain how blocking antibodies might work. Zeiss investigated the induction of IgG to ragweed allergen and the extent to which this could block binding of allergen to IgE.12 IgG concentrations increased substantially during immunotherapy, and the ratio of IgG to IgE allergen binding was found to correlate with clinical scores. IgG4 was shown to have specific allergen blocking properties and the ratio of IgG4 to IgG was also found to correlate with clinical score.7 IgG4 antibodies also have unique features that grant them anti-inflammatory properties. For example, they are able to switch the Fab arms to produce bispecific antibodies that are unable to form immune complexes or activate complement.14 Other factors that might be involved include the specificity and affinity of the antibodies concerned. Hence, IgG antibodies are now regarded as genuine functional contributors to the mode of action of immunotherapy.6 Rather than simply “mopping up”
allergens, blocking antibodies are now known to prevent IgE allergen complexes from activating immune cells, and can directly ameliorate allergic inflammation. 

A number of studies have demonstrated the induction of IgG antibodies in dogs during immunotherapy. Hites developed an ELISA to show that allergen-specific IgG increased for a number of allergens. Dermatophagoides farinae-specific IgG antibodies to multiple proteins, including the high molecular weight allergen Der f 15 were also significantly increased in dogs undergoing ASIT with aqueous allergens, but no consistent changes in total DF-specific IgG or IgG subclasses were observed following treatment with alum precipitated vaccines.

**Suppression of effector cells**

Immunotherapy is known to have effects on the eosinophil, basophil and mast cell populations. In humans, there is a decrease in the number of eosinophils and basophils in the nasal mucosa of hay fever sufferers following immunotherapy. This is associated with a reduction in the cytokines responsible for the proliferation of these cells such as IL-5. The number of mast cells in the skin is also reduced approximately seven-fold following successful immunotherapy. The mechanisms for how this is achieved are currently not understood but could involve reduction in the production or release of bone marrow precursors, inhibition of migration from blood to tissues, impaired maturation, or decreased survival. All of these processes involve the interaction of the mast cell receptor c-kit and the cytokine stem cell factor, along with the cytokine IL-9. The latter cytokine is reduced in nasal mucosa following immunotherapy, and it may account for the reduced migration of mast cells into this tissue during pollen season.

There is also a very rapid decrease in the susceptibility of mast cells and basophils to degranulation, even though IgE levels are unaltered. Early studies did not uncover the mechanism underlying this change but more recent studies have indicated that the rapid change in responsiveness to allergens may be due to upregulation of Type 2 histamine receptors which appear able to suppress IgE-mediated activation of basophils via the FcεR1 receptor.

**Effect on cytokines and T cell populations**

Once it became known that T cells were critically important in the development of allergic diseases, they became an obvious target for studies on the efficacy of immunotherapy. The discovery of the polarisation of T helper cells into TH1 and TH2 sub-types based on their cytokine profiles provided a new paradigm for the pathogenesis of atopic disorders. A number of studies have demonstrated that T cell proliferation in response to allergen challenge is reduced following immunotherapy. For example, when peripheral blood mononuclear cells from bee venom sensitive individuals were stimulated with the major bee venom allergen phospholipase A2, the proliferation index decreased significantly after 50 days of immunotherapy. In contrast, no decrease in lymphocyte proliferation was seen with the irrelevant antigen tetanus toxoid.

Numerous studies have also demonstrated a shift in cytokine production from TH2 to TH1, with decreased production of IL-4 and IL-5 and increased production of IFN-γ. These changes would be expected to result in lower concentrations of allergen-specific IgE (which does not occur consistently during immunotherapy), reduced numbers and activities of eosinophils (does occur during immunotherapy) and increased production of IgG (does occur during immunotherapy).

Of more recent interest has been the role of IL-10 and regulatory T cells in the mechanism of action of immunotherapy. Bellinghausen and colleagues were the first to describe the production of IL-10 in response to bee venom immunotherapy. Subsequent studies confirmed this finding and also demonstrated that IL-10 was important in the natural tolerance to bee stings seen in bee keepers, a phenomenon attributed to anergy. IL-10 production has also been demonstrated following conventional grass pollen immunotherapy. IL-10 has numerous anti-allergic effects including modulation of IL-4 induced B-cell production of IgE towards IgG4; inhibition of IgE mast cell activation; inhibition of IL-5 production and eosinophil survival and activation; and induction of anergy.

**Regulatory T cells**

The role of the regulatory T cell is now considered pivotal in the mechanism of action of immunotherapy. There are two types of CD4+ regulatory T lymphocyte – the natural regulatory T cell (nTreg) and the induced regulatory T cell (iTreg). Natural regulatory T cells develop as a distinct CD4+ lineage in the thymus whereas iTregs develop from conventional naïve T cells that are already in the periphery. Together, these regulatory T cells make up about 10% of the circulating T cell pool. Regulatory T cells are characterised by the presence of CD4, CD25 (IL-2 receptor) and expression of the forkhead/winged helix transcription factor Foxp3. Natural regulatory T cells start to express Foxp3 within the thymus as single positive CD4+ lymphocytes, a process that is dependent on TCR recognition of self-peptides, and stimulation by IL-2 and possibly TGF-β. Induced regulatory T cells develop in the periphery in
response to various signals, including low dose antigen, IL-2, TGF-β and retinoic acid. CD4+, CD25+, Foxp3+ regulatory T lymphocytes have been characterised in the dog.

Regulatory T cells play a pivotal role in the development of tolerance and protection of the body from autoimmunity, as well as regulating the magnitude of immune responses to infectious agents and tumors. One of the key ways in which they achieve this is via the production of the inhibitory cytokines IL-10, TGF-β and IL-35. Many of the earlier described immunological changes that occur during immunotherapy can be initiated and coordinated by the cytokines produced by regulatory T cells. Both types of regulatory T cell are thought to be involved in the induction of peripheral T cell tolerance that occurs during immunotherapy. The development of peripheral regulatory T cells during immunotherapy is thought to be controlled by dendritic cells which release various tolerogenic signals.

Clinical improvement in hay fever sufferers following immunotherapy was accompanied by an infiltration of the nasal mucosa with CD4+, CD25+, Foxp3+ regulatory T lymphocytes. A shift towards IL-10 producing Treg cells away from TH2 cells is also seen during immunotherapy. The major changes induced by regulatory T cells in tolerant healthy individuals, or allergic patients undergoing immunotherapy, include suppression of Th1, Th2 and Th17 cells; suppression of eosinophils, mast cells and basophils; Ab isotype change from IgE to IgG4; suppression of inflammatory dendritic cells; and suppression of inflammatory cell migration to tissues.

**Regulatory B cells**

In addition to the well-known regulatory T cell populations, there is also a sub-population of B cells known as Breg. The existence of these cells has been known for some time, but they received relatively little attention until it was shown that certain autoimmune and inflammatory conditions became much worse in B cell deficient mice or B cell depleted patients. As with regulatory T cells, these cells are also able to produce IL-10, TGF-β and IL-35 and can exist at various stages of maturation ranging from immature B cells through to plasma cells. IL-10 producing Bregs are potent inhibitors of T cell proliferation and produce vastly more IgG4 than other B cell types. It is now known that Bregs are induced by allergen specific immunotherapy and they likely contribute to the successful clinical outcomes achieved by the immunological changes described earlier.

**Summary**

Various immunological changes are induced by allergen specific immunotherapy, all of which may contribute to the overall success. These include:

- Increased specific IgG4 levels in serum
- Decreased numbers of mast cells, eosinophils, basophils and their mediators
- Decreased allergen-specific T-cell proliferation
- Decreased TH1 and TH2 cytokine levels
- Increase in Treg and Breg cells in circulation and tissues
- Increased IL-10 and TGF-β levels
- Decreased clinical and experimental late-phase responses
References


61. van de Veen W. The role of regulatory B cells in allergen immunotherapy *Current Opinion in Allergy and Clinical Immunology* 2017;17:447-452.

Cheilitis-a forgotten or inadequately described, sign of canine atopic dermatitis.
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Introduction
Cheilitis was considered a minor diagnostic sign of Canine Atopic Dermatitis (CAD) in Willems' proposed criteria. Prelaud found cheilitis to be a significant clinical sign of CAD and included it in his five diagnostic criteria. In a major study of diagnostic criteria for CAD Favrot et al described two sets of diagnostic criteria for CAD based on an analysis of specificity and sensitivity of numerous signalment, history, clinical signs and allergy test characteristics of 843 dogs. Cheilitis (lip lesions) was one of the clinical signs but did not feature in either of the two sets of diagnostic criteria. The lip consists of the labial mucosa, the lips margin and skin. None of the above studies specified which areas of the lip had been examined.

The study
This is a prospective study of signs of inflammation of the labial mucosa of the upper lip in the area between the first premolars. 105 anaesthetised dogs with no clinical signs or history of dermatitis were examined in two general practices (98 by Dr Gordon). The dogs were anaesthetised for other procedures. Over 80 CAD dogs sedated for intradermal allergy testing were examined by a dermatologist (Dr Simpson). Lesions were recorded on a scale of 0-4 and photographed. The scoring was reviewed on the basis of the photographs (Dr Bell). Age and breed were recorded for all dogs.

Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Young -3.5y/m</th>
<th>Middle 3.6-6.9</th>
<th>Old &gt;7</th>
<th>Presence of lesions</th>
<th>Number/Grade 0-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal dogs</td>
<td>29</td>
<td>26</td>
<td>49</td>
<td>3 dogs</td>
<td>2/1, 1/2</td>
</tr>
<tr>
<td>105</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atopic dogs</td>
<td>49</td>
<td>19</td>
<td>12</td>
<td>All dogs</td>
<td>23/1, 32/2, 25/3</td>
</tr>
<tr>
<td>&gt;80</td>
<td></td>
<td></td>
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</tbody>
</table>

Breeds
There were 4 or more Labrador Retrievers, their crosses, Poodles and Golden Retrievers amongst the "normal" dogs. Labradors, their crosses and Shih Tzu were the most common among the atopic dogs.

Conclusion
Signs indicating inflammation of the labial mucosa (cheilitis) were present in all atopic dogs suggesting a high sensitivity. No conclusions could be made for specificity in terms of CAD but the condition was very uncommon in dogs with a normal skin. The two groups were not age matched.

Discussion
This is a small single-city study. The findings here need to be repeated in more locations. Favrot recorded a sensitivity of 0.418 and a specificity 0.719 for lip lesions in his multi-continental study. However given the high sensitivity of this clinical sign in our study we think cheilitis should be reconsidered and its specificity for atopic dermatitis established. Such studies may result in a set of CAD criteria with improved sensitivity and specificity.

References
Biological therapy involves living organisms, substances from living organisms, or laboratory-produced versions of such substances. Many biologics such as vaccines, allergen immunotherapy or recombinant hormones have been used in veterinary medicine for many decades. Therapeutic monoclonal antibodies (mAb), a therapy pioneered in human medicine in the last 30 years, are now reaching veterinary field where they present the potential for targeted treatment of different diseases including allergies, pain or neoplasia. Therapeutic mAbs offer benefits over conventional therapy in terms of potency, dosing frequency and, due to the specific targeting, potential. Of course, the latter is dependent on the nature of the target and its role in the living individual. This 30-minute presentation will review the structure of mAbs, their mechanism of action and metabolism, and it will discuss mAbs that have been tested and/or are used in dogs with atopic dermatitis. An excellent review on mAbs was recently published and will serve as the notes for this lecture as it is comprehensive and up-to-date (accessible free online).

References:


