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Fort Dodge

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This year’s annual Dermatology Chapter meeting is again held during Science week at the Gold Coast, Queensland, which remains a fabulous forum for presentation and discussion of new and exciting areas of research and the most up-to-date knowledge in a range of veterinary specialties. In the Dermatology stream this year we have two full days of presentations so well done Linda on working so hard on obtaining such world class speakers.

The first day covers immune-mediated skin disease with a focus on Pemphigus and Basement Membrane Zone (BMZ) diseases, exploring similarities and differences between manifestations in people and our feline and canine patients. Our major speakers include Dr Lizbeth Intong from Sydney’s St George Hospital who is specialist human dermatologist with research interests in this area and the well known veterinary international dermatologists Dr Toshiroh Iwasaki and Dr Peter Hill (although we now claim Peter as Australian!). Together they have a wealth of knowledge and experience in the diagnosis and management of immune mediated skin diseases in people and animals respectively. They will present current ideas on pathogenesis and interesting research data on disease management. An exciting list of supporting local veterinary dermatologists, small animal medicine specialists and residents will cover therapeutic options of glucocorticoids, azothioprine, chlorambucil; with a good dose of clinical pharmacology thrown into the mix. Special thanks to Dr Graham Swinney for crossing into the cutaneous realm for an update on an internists approach.

The second day commences with Erythema Multiforme EM (the great masquerader), Toxic Epidermal Necrosis TEN (a nasty disease), Stephens-Johnson Syndrome SJS (perhaps a new name for some) including the latest information about diagnosis and management. Again Dr Intong will speak of her experience in humans, and Drs Bell and Hill will present their veterinary dermatologist experiences in dogs and cats. The remainder of day two is dedicated to newer therapeutic options such as IV immunoglobulin therapy and non-steroidal anti-inflammatory therapy. A team of veterinary dermatologists will chair the open forums on both Days 1 and 2 focusing on formulations, protocols, dose refinement, monitoring and challenges. These are always great interactive discussions.

Once again we are very excited about this year’s presentations, bringing together an array of experts from different fields to expand our knowledge and stimulate new ideas in these areas. We expect our program will appeal to dermatologists and medical specialists in veterinary referral practice, dermatology or small animal medicine residents in training and any veterinary practitioner with an interest in practical and useful take home information about the diagnosis and management of immune mediated skin diseases in their patients.

With thanks
Amanda Craig
Linda Vogelnest
Peter Hill
(Dermatology Chapter Science Week coordinators)

Acknowledgement: We would like to extend our sincere gratitude to Mandy Burrows for her contribution in organising sponsorship for the scientific programme and Rebecca Basset for passing on the wisdom of a previous Science Week organiser.
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Speakers

Dr. Fiona Bateman  
BVSc (Hons), MACVSc (Small Animal Medicine)

Fiona graduated with honours from the University of Melbourne in 2005. Following completion of a rotating Internship at Queensland Veterinary Specialists and Pet Emergency in 2008, she accepted a residency position with Dr. Mike Shipstone at Dermatology for Animals. Fiona was awarded Membership of the Australian College of Veterinary Scientists in 2009 in Small Animal Medicine, and is planning to undertake both Fellowship and Diplomate examinations in Veterinary Dermatology.

Allan Bell  
BVSc, MACVSc (Canine Medicine), FACVSc (Dermatology)

Allan graduated from Massey University in 1967 and worked in companion animal practice until the mid 80s. He became a member of the Australian College by examination in Canine Medicine in 1981 and attained a Fellowship in Dermatology by examination in 1995. He is a registered specialist and has a referral dermatology practice based in Auckland. He is first author of six papers published in refereed journals and was an invited speaker at the 1999 World Small Animal Association Congress. He is a regular contributor to Continuing Education in dermatology and speaker at the ACVSc meeting. He runs a small deer farm and enjoys sailing.

Dr Greg Burton  
Greg graduated from the University of Queensland in 1983. After spending several years in general practice he passed his Membership of the Australian College of Veterinary Scientists in Small Animal Surgery. Greg became a Fellow of the College in Veterinary Dermatology in 1997. From 1999 to now Greg has been a director of the Skin, Ear and Allergy Service at the Melbourne Veterinary Specialist Centre. In 2002, he became the Principle Fellow in Dermatology at the University of Melbourne in addition to taking responsibility for teaching of under-graduate dermatology in the veterinary faculty. Greg has authored and co-authored numerous articles in both Australian and international journals.

Peter Hill  
Peter is a UK graduate with 23 years experience of both general practice and specialist referral work. After spending 5 years in a UK small animal hospital, he trained as a dermatologist in the USA and then worked in dermatology referral centres at the Universities of Edinburgh and Bristol, picking up a PhD on mast cell biology on the way. Peter is the only veterinary dermatologist in the world to have been board certified as a Diplomate in the UK, Europe and the USA, as well as having a PhD. He has trained 9 dermatology residents, written a textbook on clinical dermatology, published over 65 papers, and given over 200 invited talks and presentations.

Lizbeth Ruth Aragones Intong  
MD  
Dermatology Fellow and Visiting Medical Practitioner  
Department of Dermatology  
St. George Hospital  
Kogarah, NSW

Dr. Lizbeth Intong completed her medical degree and dermatology training at the University of the Philippines-Philippine General Hospital and is a diplomate of the Philippine Dermatological Society. She completed one year of dermatology fellowship at Changi General Hospital in Singapore prior to doing two years of dermatology fellowship at St. George Hospital here in Sydney. She has authored several book chapters on molecular biology, epidermolysis bullosa and drug reactions in children. Her interests include bullous diseases, pediatric dermatology, dermatitis and clinical research.
Dr Toshiroh Iwasaki

Dr Iwasaki graduated from Veterinary School at the Tokyo University of Agriculture and Technology in 1974, completed a PhD at the University of Tokyo in 1983, and became a Diplomat of the Asian College of Veterinary Dermatology in 2005. He has held numerous research positions in veterinary and medical dermatology schools in Japan and the USA since 1974, and has been author or co-author of 36 peer-reviewed research and clinical publications since 2005.

Dr Iwasaki has a prominent role in the international dermatology forum, as president of the 6th World Congress of Veterinary Dermatology, and current president of the Asian College of Veterinary Dermatology. He is currently Professor at the Department of Veterinary Internal Medicine at the Tokyo University of Agriculture and Technology, and at the forefront of some areas of dermatology research including immune-mediated diseases and barrier function defects.

Dr Philippa Ravens

Philippa graduated with honours from the University of Sydney in 2004 and worked in small animal general practice in Sydney for five years. She accepted a veterinary dermatology residency in August of 2008 at the University of Sydney Veterinary Teaching Hospitals and sees both small and large animals at the Camperdown and Camden clinics. Philippa is planning on undertaking the Fellowship examinations in Veterinary Dermatology in 2013.

David Robson

BVSc (hons), MACVSc (Canine Medicine), FACVSc (Dermatology)

David graduated in 1992 from the University of Queensland. He worked as an associate at Chermside Veterinary Hospital, Brisbane, for six years and gained Membership to Australian College of Veterinary Scientists in Medicine of Dogs in 1999. Later that year he took up residency at the Animal Skin Ear and Allergy Service at the Melbourne Veterinary Referral Centre, Glen Waverley, under the supervision of Greg Burton, and passed his ACVS Fellowship examination in dermatology in 2005. His major publications include a two-part review on cyclosporine and its role in dermatology, the cytology of the normal anal sac, and the longest case series to date of *Demodex injai* demodicosis. He has practiced clinical dermatology and lectured both in Australia and overseas.

Mike Shipstone

BVSc (hons), MACVSc (canine medicine), FACVSc (dermatology), DipACVD

Michael graduated from Queensland University in 1984 and has worked in a number of different private practice and industry positions. In 1995 he started a residency at the Animal Skin and Allergy Clinic under the mentorship of Drs Ralf Mueller and Sonya Bettenay, with additional periods of study at the University of California, Davis and Louisiana State University, Baton Rouge.

Mike is a Fellow of the Australian College of Veterinary Scientists (Veterinary Dermatology) and a Diplomate of the American College of Veterinary Dermatology.

He has published in Australia and overseas and has presented both in Australia, Asia and North America.

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Dr Graham Swinney

BVSc, DVCS, FACVSc

Dr Graham Swinney graduated from The University of Sydney in 1987. He then took up a position at Massey University Dept of Veterinary Clinical Sciences as an Assistant Lecturer in Small Animal Medicine, where he spent three years. From there he spent two years at the University of Queensland as a Resident in Small Animal Medicine. In 1993 Graham returned to the University of Sydney as a Registrar and subsequently Senior Registrar in Small Animal Medicine gaining his FACVSc in Canine Medicine in 1998. Then in 1999 Graham took up the position as Clinical Assistant Professor Small Animal Internal Medicine Department of Veterinary Clinical Sciences, Washington State University. In August 2005 he returned to Sydney and worked in private practice at the Veterinary Specialist Centre in North Ryde. Since 2007 Graham has also acted as a part time internal medicine consultant for IDEXX Laboratories in Sydney.
Graham returned in September 2009 to the University Veterinary Teaching Hospital Sydney as a canine medicine specialist, where he has also been hospital superintendent since March 2010.
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Pathogenesis of pemphigus: past, present and future

Toshiroh Iwasaki, DVM, PhD, Dip AICVD
Department of Veterinary Internal Medicine, Tokyo University of Agriculture & Technology
Tokyo, Japan

1. Structure of attachment apparatus of epidermal keratinocytes

Epidermal keratinocytes attach to neighbouring cells to strengthen epidermal tissue integrity. The major apparatus for this adherent system is the desmosome. Desmosomes are discoid junctions with a diameter of 0.2–0.3 µm and are composed of two electron-dense plaques in each of the two adjacent cells, which are separated by an intercellular cleft of 24–30 nm. Desmosomes contain proteins from at least three protein families. Desmosomal cadherins form the intercellular adhesive interface, while armadillo and plakin family proteins build up these plaques. The intracellular termini of desmogleins (Dsg) and desmocollins (Dsc) interact with plakoglobin that in turn binds to desmoplakin. Finally, desmoplakins are anchored to the intermediate cytokeratin. These interactions appear to be stabilised by plakophilin.

Desmosomal protein members of the cadherin superfamily (Dsg 1–4) and desmocollins (Dsc 1–3) are single-pass transmembrane glycoproteins, which mediate adhesion in a Ca\(^{2+}\) dependent manner. The amino-terminal extracellular domain of desmosomal cadherins consists of four cadherin repeats (EC1–4) of approximately 110 amino acids followed by a less related membrane-proximal domain (EC5). The EC1–4 domains are rigidified by binding up to three Ca\(^{2+}\) ions each. The cytoplasmic domain contains a juxtamembranous anchor (IA) region that possesses a desmoplakin-binding element.

Figure 1: Ultrastructural view of desmosomes
N: nucleus, D: desmosome, cm: cell membrane
2. Definition of pemphigus in human medicine

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

3. Clinical aspects of pemphigus in dogs

The first case of canine pemphigus foliaceus (PF) was described by Halliwell et al. in 1977, two years after the first report of canine pemphigus vulgaris (PV) by Hurvitz et al. and Stannard et al. Among many publications of sporadic PV and PF cases, Scott et al. compared 11 PV and 7 PF cases from dogs with human pemphigus that revealed numerous analogies between these diseases. To the date, there have been four articles reviewing a large number of canine PF cases, with two suggesting that there may not be sex predilection, but a breed predilection in the Akita. In fact, the Akita is a breed of Japanese origin and we see few PF cases in Akita; therefore, other factors may exist for breed predilection.

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

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

In canine PV and PF, loss of attachment between epidermal keratinocytes, acantholysis, has been suspected to be related to the reaction of antibodies with specific trans-membrane desmosomal proteins, Dsg3 and Dsg1. However, while nearly 100% of dogs with PF that have not been treated with immunosuppressive drugs have IgG deposits in lesional skin, less than 5% of canine cases have circulating antibodies detected by indirect immunofluorescence. This is in contrast to human patients who have circulating antibodies in more than 90% of cases with PF. Various attempts to improve the detection of circulating antibodies in a patient with canine PF have been conducted by indirect immunofluorescence test (IIF). Suter et al. demonstrated that a reactive 148 kDa protein in canine lip epithelium was detected by western immunoblot using two canine PF sera. This 148 kDa protein was thought to be Dsg1. Another immunoblotting study showed reactivity to a 160 kDa protein in the extracts of cultured keratinocytes recognised by eight out of 16 sera with canine PF. These 148 and 160 kDa proteins are both thought to be...
canine Dsg1 but represent differentially glycosylation forms. Furthermore, one study also found that canine PV serum detected a 130 kDa protein in the extracted proteins from canine cultured keratinocytes.

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

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

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

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

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

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

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

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

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

8. Present perspectives for the diagnosis of pemphigus

Canine PV is an autoimmune skin disease whose target protein is Dsg3. The diagnosis of PV is able to be performed by the combination of findings as follows: 1) clinically, mucous membrane lesions are predominant; 2) histologically, separation of the epidermis at a supra-basal layer with acantholytic cells; 3) IgG deposition at the intercellular space of the epidermis by direct and indirect IF; and 4) a non-invasive test to detect circulating autoantibodies by ELISA using recombinant canine Dsg3.

The pathogenesis of the majority of canine PF is not known except that some PF sera can recognise canine Dsg1. Therefore, it is possible that canine PF might be a group of diseases caused by different
mechanisms. However, canine PF shows common clinical signs, histopathology, and IgG deposition in the intercellular space of epidermis. Therefore, we may be able to diagnose canine PF by classical clinical signs, subcorneal pustules with numerous neutrophils/eosinophils and acantholytic cells.

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

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

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

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Pemphigus foliaceus: review of clinical signs & diagnosis in dogs and cats

Dr. Peter Hill BVSc PhD DVD DipACVD DipECVD MRCVS MACVSc
Veterinary Specialist Centre, North Ryde

Classification and clinical signs of pemphigus in dogs and cats
Pemphigus can broadly be classified into superficial and deep forms

Pemphigus foliaceus
The most common autoimmune skin disease of dogs and cats.
Lesions progress from erythematous macules to papules to pustules to crusts.
N.B. Compared to pyoderma, PF pustules are usually more numerous, larger, may have irregular, highly erythematous borders and may coalesce.
Distribution is usually facial/pedal or may be generalised (Figure 1).

Figure 1 - Lesion distribution patterns in pemphigus foliaceus

Pemphigus erythematosus
Probably a photosensitive form of pemphigus foliaceus primarily restricted to the face.

Panepidermal pustular pemphigus (pemphigus vegetans)
May be a variant of PF, although the pustules may be deeper.
Lesions are similar to PF but may become verrucose.
Lesions are clinically the same as facial PF.

Pemphigus vulgaris
The rarest form of pemphigus.
Lesions are erosions and ulcers of the muco-cutaneous junctions and oral cavity. Vesicles are rarely seen due to the fragility of the canine epidermis. If they are seen, it is likely to be in the oral cavity.

Paraneoplastic pemphigus
Rare form of pemphigus.
Has been seen in association with tumours such as thymic lymphosarcoma, mammary carcinoma, Sertoli cell tumour.
Histologically, the lesions can show features of both PV and erythema multiforme.
Drug-induced pemphigus
Some cases of PF are thought to have been induced by drugs.

Pemphigus of chronic disease
Some authors believe that dogs that have suffered from other skin diseases for prolonged periods of time (allergy, chronic pyoderma) may develop pemphigus later in life. There is currently no evidence to support this anecdotal observation.

Diagnosis of pemphigus
As with other skin diseases, the diagnosis of autoimmune skin diseases relies on history, physical examination and diagnostic tests (cytology and histopathology).

History
The history of dogs and cats with autoimmune skin diseases is usually non-specific. Due to the severity of many of these diseases, owners usually present their animals as soon as the disease appears. With some autoimmune skin diseases such as pemphigus foliaceus, the lesions may appear in waves and go through periods of waxing and waning. Systemic manifestations such as lethargy and anorexia are variable. Some people believe that dogs that have suffered from recurrent skin disease (especially allergic disease and pyoderma) for a long time can ultimately develop a form of pemphigus foliaceus. A true cause and effect has yet to be proven.

Physical examination
The main objectives of the physical examination are to determine:
1. The nature of the skin lesions – are they superficial and pustular/crusting or are they deep and erosive/ulcerative.
2. The distribution of the skin lesions – are they confined to certain body regions such as the face or mucocutaneous junctions or are they generalised.
3. Are there any other abnormalities such as joint swelling, anaemia etc.

Based on the physical examination alone, it is possible to draw up a list of differential diagnoses that includes or excludes some of the above autoimmune skin diseases, as well as other, non immune-mediated diseases. However, without further diagnostic tests, it is not possible to make a specific diagnosis.

Diagnostic tests
The two most useful tests in the diagnosis of autoimmune skin diseases are cytology and histopathology.

Cytology
Cytological examination should be performed in all cases of suspected autoimmune skin disease. In cases of pemphigus foliaceus (and the clinically similar variants), cytology can be virtually diagnostic because of the presence of acantholytic keratinocytes (Figure 2). In all autoimmune skin diseases, cytology can help to confirm the presence of secondary bacterial infection or other differential diagnoses.
Figure 2 – Microscopic field showing the cytological appearance of PF

Acantholysis refers to a process in which the keratinocytes from deeper layers within the epidermis become separated from each other. The separated cells are known as acantholytic keratinocytes or acanthocytes. Acanthocytes are very large cells with a centrally placed nucleus that are about 3-5 times the diameter of a neutrophil. The presence of large numbers of acanthocytes amongst a background of neutrophils is a characteristic finding in pemphigus foliaceus. However, culture of pustules and biopsy is indicated whenever this picture is seen because bacterial infections can occasionally cause this pattern of inflammation (X400).

Biopsy and Histopathology

Although cytology is a very useful test that can be performed in the clinic, it is essential to biopsy all cases of suspected autoimmune skin diseases. With the appropriate samples it may be possible to make specific diagnoses of pemphigus foliaceus, pemphigus erythematousus, panepidermal pustular pemphigus, pemphigus vulgaris, and cutaneous lupus. It is not possible to distinguish between bullous pemphigoid, mucous membrane pemphigoid, linear IgA bullous dermatosis and epidermolysis bullosa acquisita on histopathology.

When taking biopsies from autoimmune skin diseases, the following tips may help to get diagnostic results:

- Biopsy punches are acceptable for the majority of lesions but ellipse biopsies may be preferable for large bullae or ulcers.
- Always take at least five biopsies.
- For pustular/crusted lesions, try and biopsy pustules. If none are present, biopsy papules or macules – they may contain microscopic pustules. If you have to biopsy crusted lesions, make sure that the crusts are submitted along with the skin.
- If you suspect pemphigus foliaceus but there are no primary lesions, clip the fur off an area of skin and wait for 24-48 hours. New lesions may develop in the clipped area (or other areas).
- If ulcers are biopsied, sample the junction between normal and ulcerated skin. Make sure that the biopsy is orientated so that the pathologist sections the skin the right way.
- If there are ulcerative lesions, rub an unaffected area of skin with a pencil eraser for about a minute. If there is dermo-epidermal weakness, this can artefactually create a new lesion to biopsy.
- If the first set of biopsies are non-diagnostic but you still suspect an autoimmune disease, biopsy the animal again.

Histopathological changes in the skin

The key histopathological findings of the various diseases can be summarised as follows:

- Pemphigus foliaceus – Intracorneal or subcorneal pustules containing neutrophils and acantholytic keratinocytes; surface neutrophilic crusts containing degenerated acanthocytes.
- Pemphigus erythematousus – Epidermal changes as for pemphigus foliaceus, but with lichenoid or interface changes at the dermo-epidermal junction. These may include hydropic degeneration of basal cells, basal cell apoptosis, abnormal appearance of the basement membrane and a sub-epidermal infiltrate of mononuclear cells, mainly lymphocytes.
- Pemphigus vulgaris – Suprabasilar acantholysis leads to cleft formation above the basal cell layer of the epidermis. The basal cells may remain attached to the basement membrane like a row of tombstones.
- Paraneoplastic pemphigus – this disease has features of both PV and erythema multiforme i.e. suprabasilar cleft formation with apoptosis at multiple layers throughout the epidermis.

Other tests that should be performed when a skin disease is suspected to be auto-immune include fungal culture (in suspected cases of PF), bacterial culture and sensitivity (in suspected cases of PF and if rods are seen on cytology), skin scrapings to rule out demodicosis, and a routine haematology and biochemistry panel to allow monitoring of therapy and to look for evidence of systemic involvement.
Immunofluorescence and immunohistochemistry

These techniques allow detection of the autoantibodies directed against skin targets. However, they are not widely available in commercial histopathology labs, although they have been used extensively in research. The principles underlying these tests are illustrated below.

**Direct immunofluorescence**

1. Skin section taken from a dog with autoimmune skin disease
2. Tissue section
3. Autoantibody or complement components to be detected (already bound to diseased skin)
4. Specific antibody labelled with fluorescent marker is added
5. Fluorescent marker visualised under UV light microscope

**Indirect immunofluorescence**

1. Skin, lip, oesophagus section taken from a normal dog
2. Tissue section
3. Serum from a dog with autoimmune skin disease containing autoantibodies to skin components is added
4. Specific antibody labelled with fluorescent marker is added
5. Fluorescent marker visualised under UV light microscope

**Immunohistochemistry**

1. Tissue section taken from an animal with skin disease (autoimmune or tumour)
2. Tissue section
3. Autoantibody or tumour-specific protein to be detected (already bound to, or part of, diseased skin)
4. Specific antibody labelled with enzyme marker is added
5. Enzyme reaction with substrate allows visualisation of specific proteins under microscope
Basement membrane zone macromolecules and related diseases in dogs

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Using light microscopy, it can be observed that the basement membrane separates the epidermis from the dermis. In reality, it actually attaches the epidermis to the dermis by several adhesion molecules when observed with an electron microscope. Humans and animals are known to suffer blistering skin diseases if at least one of these adhesion molecules is impaired. In this section, the normal fine structure of the basement membrane zone is explained and disorders caused by disorganisation of adhesion molecules are reviewed.

1. Normal fine structure of cutaneous basement membrane zone

Electron microscopic analysis of the epidermal basement membrane zone (BMZ) reveals that it is made up of an interface between keratinocytes and the dermis. At higher magnifications, there are several structures within the BMZ. At the dermal side of the basal keratinocyte, electron dense structures are seen, called hemidesmosomes (HD), which exhibit stud-like structures at the site of the plasma membrane. HDs are subdivided into an inner and outer plaque, with the outer plaque overlapping with the cell membrane. An electron lucent area is observed just beneath the plasma membrane of basal keratinocytes, where fine fibrillar materials known as anchoring filaments, traverse the lamina lucida, has been identified. The lamina densa is an electron-dense band-like structure, which runs parallel to the lamina lucida. Anchoring fibrils in a looping structure beneath the lamina densa extend their loops to the dermis to hold the dermal collagen or elastin network. The HD-anchoring filament complex forms a continuous link between the basal keratinocyte intermediate keratin filaments and the BMZ and dermal components.

2. BMZ macromolecules

2.1. Keratin intermediate filaments

The basal keratinocyte filaments are composed of cytokeratins 5 and 14. Intermediate filaments are small 50–55 kDa proteins that give shape and structural integrity to basal keratinocytes. The intermediate keratin filaments act as a network through the desmosome and HD for adjacent keratinocytes.

![Figure 1: Ultrastructural view of basement membrane zone](image)

E: epidermis, D: dermis, Arrow: anchoring fibrils

2.2. Plectin

The 120 kDa plectin protein is an important molecule in the inner plaque of HDs. Plectin belongs to the plakin family and provides a link between keratin intermediate filaments in basal keratinocytes and HDs. It assists with the attachment of integrin α6β4 and collagen XVII to the outer plaque of HDs.
2.3. Bullous pemphigoid antigen 1 (BPAG1)
BPAG1 is also a member of the plakin family with a molecular weight of 230 kDa in human bullous pemphigoid patients. BPAG1 is expressed in dermal nerves and basal keratinocytes. In the basal keratinocytes, BPAG1 is attached to keratin intermediate filaments, the intracellular domain of collagen XVII (BPAG2) and the cytoplasmic domain of integrin β4.9

2.4. α6β4 integrin
The α6β4 integrin expressed in the HD plaque complex is the chief receptor of laminin-332 in the lamina lucida. A sub-type of human junctional epidermolysis bullosa (Pyloric atresia type JEB), it demonstrates the lowest level of expression of all α6β4 integrins.9 The α6β4 integrins also bind to collagen XVII, BPAG1 and BPAG2 in the lamina densa. Collagen VII has been proposed to bind to α6β4 integrin via small proteins, which have been identified as fibulin 2. There have been no reports of clinical cases caused by the genetic dysregulation of α6β4 integrin in canine JEB.

2.5. Laminin-5 (laminin α3β3γ2, laminin-332)
Laminin-332 is the primary component of anchoring fibrils in the lamina lucida and is composed of three chains, α3, β3 and γ2. Laminin-332 plays a crucial role in the BMZ network as one of the main components of adhesion between the epidermis and dermis.1 The long arm of the α3 chain has globular domains (LG1–5) at the C-terminal, however, during processing, the short arm of the γ2 chain and the LG4 and LG5 domains are cleaved by enzymes.1 Laminin-332 attaches to the NC1 domains of type VII collagen in the scaffold of collagen IV by short-arm β2 and γ2 chains, collagen XVII at the lamina lucida/densa by the γ2 chain, and integrin α6 by the LG1–3 domains at the lamina lucida.

2.6. Collagen XVII (BP180, BPAG2)
Type XVII collagen (formerly known as BP180 or BPAG2) is a transmembranous protein with an extracellular domain that spans the entire length of the lamina lucida, and gives stability to anchoring fibrils. Canine BPAG2 has been characterised as the canine counterpart in humans with 85% identity at the deduced amino acid sequence level.13,15 The extracellular domain of BPAG2 contains an NC16A non-collagenous domain just beneath the cell membrane. An NC16A domain, targeted by patient sera with canine bullous pemphigoid.13,15 The intracellular domain of collagen XVII binds to the α6β4 integrin and BPAG1, and the extracellular domain is anchored to the γ2 chain of laminin-332 in the lamina lucida/densa.9

2.7. Collagen IV
The classical type IV collagen in the skin BMZ is composed of a trimer [α1(IV)2α2(IV)] with a C-terminal NC-1 domain adjacent to a flexible 400 nm long interrupted triple helical domain. The heterotrimeric collagen IV molecule can form dimers via its NC-1 domain and tetramers via the N-terminal. The scaffolds of collagen IV bind to the laminin-332 group by the small adhesion molecule, nidogen.9 No skin diseases have been found to be caused by autoimmunity or genetic dysregulation of type IV collagen.

2.8. Collagen VII
Collagen VII is the main component of dermal/epidermal anchoring fibrils. A collagen VII monomer is composed of three identical central triple helical collagenous segments with repeating Gly-X-Y amino acid sequences and a large 145 kDa N-terminal, NC-1, and C-terminal, NC-2 domain at both ends.2 Cleavage of the NC-2 domain occurs by proteolytic enzymes after two monomers form an anti-parallel dimer with central overlap of the NC-2 domain with the outward NC-1 domain. Both NC-1 domains attach to the lamina densa and collagenous domains to make a loop with the dermis and “anchoring fibrils” to entrap dermal collagen fibrils. Then, covalent stabilization of the dimer by disulfide bonds occurs prior to non-staggered lateral association of the dimers to anchoring fibrils.2 Collagen VII binds to laminin-332 and α6β4 integrins by the amino-terminal NC-1 domain in the lamina densa. The NC-1 domain of canine type VII collagen was cloned and sequenced, and found to have 86% identity to the human form.1,6
3. Diseases caused by the disruption of BMZ molecules

Diagnosis of sub-epidermal blistering diseases is based on light microscopy, electron microscopy, immunofluorescence (IF) with and without salt-split skin (SSS) and immunoblotting. Direct immunofluorescence using SSS distinguishes epidermolysis bullosa acquisita (EBA) from bullous pemphigoid (BP). Perilesional skin incubated in cold 1 M NaCl for 48 h is separates the dermis and epidermis, which effectively places the BP antigen on the epidermal roof and the EBA antigen on the dermal floor.

3.1. Bullous pemphigoid (BP)

BP in a dog was first reported by Kunkle in 1978. Since then, more than 10 dog BP cases have been published, but BP is still an extremely rare autoimmune skin disease in dogs. Animals other than dogs affected by BP include cats, horses and pigs. In dogs, lesions are characterized by erythema, vesicles or bulla, followed by erosions and crust formation in the oral cavity, concave pinnae and lips. There are no reports in patients of organs other than the skin and mucous membranes being affected. The autoantigen of canine BP was demonstrated to be the 180 kDa BPAG2. The extracellular domain of BPAG2, NC16A, is the target antigen of the autoantibody in BP patients. Diagnosis of canine BP is based on clinical signs, sub-epidermal bulla or cleft formation of the dermal-epidermal junction along with eosinophilic and/or neutrophilic infiltration. Direct and indirect IF may reveal IgG deposition at the dermal-epidermal junction. However, we cannot differentiate EBA from BP by an IF test, as both diseases show positive reaction at the BMZ. Direct IF using SSS exhibits fluorescence at the roof of clefts in lesioned skin of BP cases. The 180 kDa proteins may be detected when patient serum and the extracted proteins from dog skin are reacted by immunoblotting. Positive direct IF using SSS enables us to diagnose canine bullous pemphigoid.

3.2. Mucous membrane pemphigoid (MMP)

MMP in dogs was identified by Olivry et al. as a localised variant of BP, characterised by ulcerations predominating in mucosae or at mucocutaneous junctions. Lesions usually occur inside the oral cavity, gingival, buccal mucosa, palate, tongue, nasal planum and periorbital region. A small number of animals are...
affected on foot pads and claws. Exanthema includes tense vesicles that evolved rapidly into ulcers and/or erosion. Histopathology of lesions in mucous membrane demonstrated sub-epithelial vesiculation with a superficial dermal band-like lymphoplasmacytic inflammation. Circulating IgG autoantibodies targeting the BMZ are detected in one third of patients with MMP by indirect IF, and a positive IgG staining result assists in the diagnosis of MMP. Indirect IF using SSS is a more sensitive immunohistochemical method that shows IgG deposition at the epidermal side of the cleft. Olivry proposed the following diagnostic criteria for MMP:

- Clinical examination exhibiting chronic mucosal or predominantly mucocutaneous vesiculation and ulceration with or without scarring.
- Histopathology showing sub-epidermal vesiculation with variable inflammation.
- Immunofluorescence that shows epithelium-fixed immunoglobulins, complement, or circulating autoantibodies binding the BMZ.

Treatment of MMP is managed by immunosuppressive doses of drugs such as prednisolone with or without azathioprine.

3.3. Junctional epidermolysis bullosa (JEB)

Epidermolysis bullosa (EB) is classified into three categories, EB simplex, JEB and dystrophic EB (DEB) as determined by the location of the cleft in BMZ. JEB is reported in dogs to have a lethal and non-lethal subtype, especially in a family of German short haired pointers. JEB is a blistering disease with cleft formation within the lamina lucida of the BMZ. JEB in humans is caused by genetic mutations in several different genes such as *lama3*, *lamb3* and *lamc2*, which code for the three chains of laminin-332. In dogs, a French group reported genetic mutations in the *lama3* gene, which codes for the α3 subunit of laminin-332, in German short haired pointers with JEB.

Clinical signs of canine JEB present as repeated blisters or vesicle formation followed by erosion and ulcer onset in infantile or very young dogs. After disruption of the vesicle/blister, scar formation and hair loss is visible on the face, concave pinnae and foot pads. Immunofluorescence using antibodies against laminin-332, plectin, collagen XVII and integrin α6β4 may reveal decreased expression of these adhesion molecules. Electron microscopy shows that separation of the dermo-epidermal junction occurs within the lamina lucida with a hypoplastic HD attachment plaque and normal-looking anchoring fibrils.

3.4. Dystrophic epidermolysis bullosa (DEB)

DEB is caused by a mutation in the gene encoding type VII collagen (COL7A1) in neonatal or very young animals. Clinical manifestations of DEB in dogs is caused by the separation of the dermo-epidermal junction at the sub-lamina densa and presents as vesicle formation, erosion and ulcers, and crust formation on the oral mucous membrane, tongue, palate and foot pads.

3.5. Epidermolysis bullosa acquisita (EBA)

EBA is caused by autoantibodies against the NC-1 domain of type VII collagen. To date, two variants of EBA have been reported, a generalised and a localised form. There have been two independent case reports of generalised EBA in Great Danes, as well as additional 12 cases in various dog breeds. Of these 12 cases, Great Danes made up 50% of the cases, implicating that this breed may have a predilection to a generalised form of EBA. Clinical signs include vesicles or pustules that rupture rapidly and show erosions and/or ulcers on the oral mucous membrane, face, axilla, groin and foot pads. Histopathology revealed sub-epidermal blistering with and without infiltration of neutrophils. Direct IF of lesioned skin shows deposition of IgG at the BMZ, and indirect IF using SSS and patient serum usually stains the bottom of a cleft. Western blotting may recognise a 145 or 290 kDa protein, known to be type VII collagen. These tests aim to differentiate EBA from BP. Olivry proposes the following diagnostic criteria for canine EBA:

- Predominant vesicular and ulcerative disease of the skin
- Microscopic sub-epidermal vesiculation upon histopathological examination of lesional skin biopsy specimens
- Basement membrane-bound autoantibodies and/or circulating autoantibodies targeting the dermal side of SSS by indirect IF
- Circulating autoantibodies targeting collagen VII (ELISA or immunoblotting)
References

16. Xu L, Chen M, Peng J, O’Toole EA, Woodley DT, Chan LS. Molecular cloning and characterization of a cDNA encoding canine type VII collagen non-collagenous (NC-1) domain, the target antigen of autoimmune disease epidermolysis bullous acquisita (EBA). Biochimica Biophysica Acta 1998; 1408: 25-34.
Pemphigus and BMZ diseases in people: presentations and management

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Autoimmune bullous diseases

Autoimmune bullous diseases are due to circulating antibodies directed against specific target antigens in the skin. The clinical presentation of the disease is usually influenced by the location of the target antigen. Antibodies directed against desmosomal proteins that anchor the keratinocytes to one another in the epidermis result in pemphigus and its variants. Due to the superficial location of the antigens, blisters are usually flaccid and often present clinically as erosions. On the other hand, antibodies directed against hemidesmosomal proteins that anchor the basal keratinocytes to the basement membrane zone (BMZ) result in pemphigoid and its variants. In this group of diseases, blistering is sub-epidermal and blisters appear tense. Diagnosis of these diseases is confirmed by histology and immunofluorescence. This review will focus on the clinical presentation of these various autoimmune blistering diseases, as well as an overview of their management.

Molecular Basis

The skin structure is maintained by secure adhesion between keratinocytes within the epidermis and between the basal keratinocytes and the underlying basement membrane. The structures that are mainly responsible for adhesion are the desmosomes between epidermal keratinocytes and the hemidesmosomes anchoring the basal keratinocytes to the basement membrane. Disruption of any of the proteins contained in these complexes leads to destabilization of the skin structure leading to blisters.\(^1,2\)

A summary of the most common human autoimmune bullous diseases is set out in Table 1, along with the target antigens, antibodies, and immunofluorescence pattern seen.

<table>
<thead>
<tr>
<th>Autoimmune Bullous Disease</th>
<th>Target Antigen</th>
<th>Location</th>
<th>Antibodies</th>
<th>DIF pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemphigus vulgaris (PV)</td>
<td>Desmoglein 3 &gt; 1</td>
<td>Desmosome</td>
<td>IgG, C3</td>
<td>Intercellular deposits around keratinocytes</td>
</tr>
<tr>
<td>Pemphigus foliaceus (PF)</td>
<td>Desmoglein 1</td>
<td>Desmosome</td>
<td>IgG</td>
<td>Intercellular deposits around keratinocytes</td>
</tr>
<tr>
<td>Paraneoplastic Pemphigus (PNP)</td>
<td>Periplakin, envoplakin, epiplakin, desmoplakin, plakoglobin, desmoglein 230-kDa BP antigen</td>
<td>Desmosome</td>
<td>IgG, C3</td>
<td>Intercellular deposits around keratinocytes and linear deposits along BMZ</td>
</tr>
<tr>
<td>Bullous pemphigoid (BP)</td>
<td>230-kDa BP antigen, Type XVII collagen</td>
<td>Hemidesmosome</td>
<td>C3, IgG</td>
<td>Linear deposits along BMZ, roof of blister in NaCl split skin</td>
</tr>
<tr>
<td>Mucous Membrane Pemphigoid (MMP)</td>
<td>Type XVII collagen, plectin, laminin 5</td>
<td>Hemidesmosome</td>
<td>IgG, C3, (IgA, IgM)</td>
<td>Linear deposits along BMZ</td>
</tr>
<tr>
<td>Pemphigoid Gestationis (PG)</td>
<td>Type XVII collagen</td>
<td>Hemidesmosome</td>
<td>C3</td>
<td>Linear deposits along BMZ</td>
</tr>
<tr>
<td>Autoimmune Bullous Disease</td>
<td>Target Antigen</td>
<td>Location</td>
<td>Antibodies</td>
<td>DIF pattern</td>
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<tr>
<td>---------------------------</td>
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</tr>
<tr>
<td>Linear IgA Disease (LAD)</td>
<td>Type XVII collagen (97-kDa and 120-kDa cleaved fragments)</td>
<td>Hemidesmosome</td>
<td>IgA</td>
<td>Linear deposits along BMZ, roof and floor of blister in NaCl split skin</td>
</tr>
<tr>
<td>Epidermolysis Bullosa (EBA)</td>
<td>Type VII collagen (anchoring fibrils)</td>
<td>Sub-basal layer</td>
<td>IgG, C3</td>
<td>Linear deposits along BMZ, floor of blister in NaCl split skin</td>
</tr>
<tr>
<td>Dermatitis herpetiformis (DH)</td>
<td>gliadin, reticulum, smooth muscle endomysium</td>
<td>Dermal papillae</td>
<td>IgA</td>
<td>Granular in dermal papillary tips</td>
</tr>
</tbody>
</table>

**Diagnostic Tests**

A routine skin punch biopsy (3–4 mm) is taken from the edge of the blister, including a portion of normal skin and this sent for haematoxylin and eosin (H&E) staining. A biopsy is helpful in making the diagnosis since it will show the level of the split (intraepidermal for pemphigus and subepidermal for pemphigoid). It will also show the inflammatory cells which are a clue to the disease. Bullous pemphigoid, for example, has a predominantly eosinophilic infiltrate, which accounts for the pruritus associated with the disease. Linear IgA disease, on the other hand, is associated with a neutrophilic infiltrate.

While doing skin biopsies, it is also important to take a perilesional biopsy for direct immunofluorescence (DIF). This method detects antibodies deposited within the patient’s tissue. Antibodies have the fluorescent dye attached. Fluorescein-conjugated antibodies directed against complement fractions (C3, fibrinogen) and immunoglobulins (IgG, IgA, IgM) are placed on frozen sections of the tissue. A positive fluorescence (graded on intensity) in a particular pattern confirms the diagnosis. The patient’s tissue may also be incubated in 1 mol/litre NaCl prior to performing the DIF. This technique induces cleavage through the lamina lucida, and is useful in differentiating autoimmune diseases with a similar DIF pattern by observing where the IgG or C3 localizes (i.e. dermal roof pattern for BP and dermal floor pattern for EBA).

Serum may also be sent for indirect immunofluorescence (IIF) which is useful as a confirmatory test. This is used to detect circulating autoantibodies. In this setting, antibodies do not have the fluorescent dye attached. This method uses serum with fluorescein-conjugated human anti-immunoglobulin against a mucosal substrate such as monkey oesophagus or rat bladder.

**Pemphigus vulgaris**

**Clinical**

- This disease involves the skin and mucous membranes
- Age 40–60 years
- HLA Associations: HLA DRB1*1454, DRB1*1401
- The oral mucosa is usually the first site of involvement, followed by the skin months later
- May be fatal if untreated
- Flaccid blisters rapidly becoming erosions
- (+) Nikolsky sign (extension of blister upon applying lateral pressure)
- Other variants: drug-induced pemphigus (penicillamine, nifedipine, captopril), pemphigus vegetans (involves skin folds)

**Histology**

- Suprabasal blister with acantholysis

*IIF - see table 1*
Management
- Systemic corticosteroids are mainstay: Prednisone 1 mg/kg/day +/- other immunosuppressives
- Azathioprine, Methotrexate, Cyclophosphamide, Mycophenolate mofetil, IVIG, Rituximab, plasmapheresis

Course and prognosis
- Common cause of death is infection due to immunosuppression needed to treat the disease

Pemphigus foliaceus
Clinical
- May be localised or generalised
- Shallow, flaccid blisters rapidly becoming scaly, crusted erosions, may coalesce into large denuded areas
- Mucous membranes generally not affected
- (+) Nikolsky sign (extension of blister upon applying lateral pressure)
- Other variants: fogo selvagem (endemic PF associated with black fly *Simulium nigrimanum* in Brazil), pemphigus erythematosus (localised to cheeks and forehead, may have (+) ANA)

Histology
- Intraepidermal blister at the granular layer with acantholysis

IF - see table 1

Management
- Topical corticosteroids for localised PF
- Systemic corticosteroids or other immunosuppressives in recalcitrant disease

Course and prognosis
- Good if therapy instituted early

Paraneoplastic pemphigus
Clinical
- This is due to an underlying malignancy (tumour antigens evoke an immune response leading to blisters)
- Most common tumours: leukaemia, lymphoma, Waldenstrom’s macroglobulinaemia, sarcomas, thymoma, Castleman’s disease
- 100% have mucosal involvement, highly variable cutaneous lesions

Histology
- Suprabasal blister with acantholysis, basal vacuolation, dyskeratotic keratinocytes

IF - see table 1
- In addition, rat bladder transitional epithelium separates it from PV and PF as desmogleins present in stratified squamous epithelium only

Management
- Systemic corticosteroids are mainstay: Prednisone 1 mg/kg/day +/- other immunosuppressives
- Azathioprine, Methotrexate, Cyclophosphamide, Mycophenolate mofetil, IVIG, Rituximab, plasmapheresis

Course and prognosis
- High mortality (75-80%) due to underlying neoplasm and medications required to treat this

Bullous Pemphigoid
Clinical
- Subepidermal blistering disease
- Age > 60 years
- May start as an urticarial eruption (very pruritic)
- Tense blisters, common locations: abdomen, flexor surfaces of forearms, inner thighs
- (-) Nikolsky sign

**Histology**
- Subepidermal blister with prominent eosinophilic infiltration

**Management**
- Topical steroids or systemic corticosteroids +/- other immunosuppressives
- Tetracycline +/- Nicotinamide, Azathioprine, Mycophenolate mofetil, Methotrexate, Cyclophosphamide

**Course and prognosis**
- Self-limited with good prognosis
- 50% enter remission within 2-6 years

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**Mucous Membrane Pemphigoid (Cicatricial Pemphigoid)**

**Clinical**
- Erosive lesions of skin and mucous membranes
- Skin involvement in 1/3 of patients, mostly mucosal
- Heals with scarring (i.e. conjunctival scarring)
- Eye involvement may lead to blindness
- Mucosal involvement may lead to dysphagia or even oesophageal stenosis requiring dilatation
- (-) Nikolsky sign

**Histology**
- Subepidermal blister with mixed inflammatory cell infiltration

**Management**
- Topical steroids or systemic corticosteroids +/- other immunosuppressives
- Tetracycline +/- Nicotinamide, Azathioprine, Mycophenolate mofetil, Methotrexate, Cyclophosphamide

**Course and prognosis**
- Chronic, progressive

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**Pemphigoid Gestationis (Herpes gestationis)**

**Clinical**
- Rare, autoimmune disease of pregnancy
- Extremely pruritic, polymorphic bullous dermatosis with urticarial plaques
- Usually starts on the abdomen spreading peripherally sparing the face, palms, soles, mucous membranes
- Exacerbations after delivery common
- Babies born to these mothers may have transient blistering after delivery
- Heals with scarring (i.e. conjunctival scarring)

**Histology**
- Subepidermal blister with eosinophilic infiltration

**IF - see table 1**
Management
- Topical steroids or systemic corticosteroids if required

Course and prognosis
- Maternal mortality rate is unaffected
- Regresses without scarring a few days to weeks after delivery
- May recur in subsequent pregnancies

Linear IgA Disease (Chronic Bullous Disease of Childhood)
Clinical
- Often in patients > 30 years; <5 years in children
- Abrupt onset of tense bullae on an inflammed, erythematous base
- Blisters often occur in collarettes or rosettes as new blisters arise in periphery of old blisters
- Oral ulcers in 50%
- Drugs implicated: vancomycin, lithium, dicofenac

Histology
- Subepidermal blister with neutrophilic infiltration

IF - see table 1

Management
- Dapsone or sulfapyridine

Course and prognosis
- Variable and unpredictable
- May remit spontaneously after 2 years

Epidermolysis Bullosa Acquisita
Clinical
- Chronic bullous disease primarily involving skin, but may also affect mucous membranes
- Common sites: trauma-prone areas of the skin - extensor surfaces of elbows, knees, ankles, buttocks
- Nail destruction and hair loss seen

Histology
- Subepidermal blister with mixed inflammatory cell infiltration

IF - see table 1

Management
- Systemic corticosteroids +/- other immunosuppressives

Course and prognosis
- Chronic disease with periods of partial remissions and exacerbations

Dermatitis Herpetiformis
Clinical
- Associated with HLA B8-DR3-DQ2
- Intensely pruritic, chronic skin disease
- Age 20-40 years
- Intensely pruritic, chronic, grouped papules and vesicles symmetrically distributed on extensor surfaces, buttocks, hairline
- Associated with gluten-sensitive enteropathy

Histology
• Subepidermal blister at level of lamina lucida
• Neutrophilic microabscesses in dermal papillae

**Management**
• Dapsone or sulfapyridine
• Gluten-free diet
• Avoid iodine and NSAIDs

**Course and prognosis**
• Persists indefinitely
• Waxes and wanes

**References**
Glucocorticoids: Pharmacology and practical implications

Greg Burton
Animal Skin Ear and Allergy Service, Melbourne Veterinary Specialist Centre

Introduction

Endogenous adrenal corticosteroids are essential for homeostasis and play a supportive role during stress responses via the hypothalamic pituitary adrenal axis. There is pulsatile glucocorticoid release during the day with increased amplitude at the start of the active period. Stress responses are superimposed over this ultradian background. Protein bound glucocorticoid (corticosteroid binding globulin and albumen) is not biologically available. Glucocorticoid activity is also regulated by 11β-hydroxysteroid dehydrogenase enzymes that convert active 11-hydroxy-glucocorticoids to their inactive 11-keto forms at a cellular level. Cellular activity of these enzymes affects ligand bioavailability however in the liver, fat and brain active glucocorticoids can be re-formed from inactive metabolites. Tissue bioavailability of glucocorticoids is also regulated by organ specific aspects eg the p-glycoprotein pump in the brain.

The use of exogenous glucocorticoids in medicine dates back to 1948 with their introduction as a therapeutic agent for rheumatoid arthritis. Glucocorticoids are powerful drugs used to modulate inflammation but their therapeutic benefits need to be balanced against detrimental physiological effects, especially in long-term use. Understanding how glucocorticoids work helps us make better therapeutic decisions when utilising these medications and also paves the way for development of more selective glucocorticoids with greater safety.

How do glucocorticoids work?

1. Corticosteroid receptors:
Glucocorticoids (GC) exert their action via glucocorticoid receptors (GR). These are intracellular and are closely related to other Class 1 steroid receptors including the oestrogen, progesterone and androgen receptors. The receptor structure is highly conserved with an N-terminal end (containing the ligand-independent activation function 1), a centrally located DNA binding domain and the C terminal end containing the ligand-binding pocket of the hormone. The DNA binding domain confers specificity for binding to the cortisol response element of DNA while binding of the cognate ligand in the C terminal end triggers conformational changes in the GR resulting in nuclear translocation. GR variants (isoforms) exist with differing transcriptional activities expressed in a tissue dependent manner. GR isoform (a) is biological active while isoform GR (b) undergoes DNA binding after cortisol activation but fails to induce transcription. Steroid resistance can occur associated with decreased expression of GR(a) or increased expression of GR(b). Also worth noting is that certain cytokines, particularly IL-2, IL-4, and IL-13, which are over expressed in sensitised atopic individuals can reduce GR ligand binding affinity in T lymphocytes AND increase GR phosphorylation inactivating the GR resulting in local resistance to the anti-inflammatory actions of glucocorticoids.

Post cortisol binding to the GR there is a covalent addition of ubiquitin that marks the GR for degradation in the proteasome. This process is cell type specific and allows for further tissue specific regulation of glucocorticoid activity.

Receptor expression is in high levels in the immune system, bone, lungs, liver, adipose tissue and brain, which helps explain the diversity of actions and side effects of glucocorticoids.

2. Mechanism of action:
Non-genomic effects are acute or immediate effects of glucocorticoids (GCs) and are mediated via membrane receptors and second messengers. By definition they can be detected within 5 minutes. Recent findings suggest that the glucocorticoid transporter (“steroid hormone recognition and effector complex”, SHREC) may be involved with transportation of GC into the cell and be involved in initial triggering of non-genomic effects followed later by genomic events.

Non-genomic effects are exerted by direct actions on membrane lipids (affecting membrane fluidity), membrane proteins (e.g. ion channels and neurotransmitter receptors), and cytoplasmic proteins (e.g.
mitogen activated protein kinases (MAPKs), phospholipases etc). These actions may be direct receptor binding OR be indirect due to proteins that dissociate after GC binding to the GR. Non-genomic effects are less well understood than the genomic effects but do appear to be clinically relevant. Specificity for non-genomic activity may occur amongst the different GCs. It is worth noting that the adverse events associated with long term GC therapy relate to genomic effects. The development of GC that specifically triggers non-genomic events would improve safety.

Genomic actions occur more slowly after hormone binding to the receptor. Ligand binding results in conformational change with detachment of the chaperone proteins (hsp70, hsp 90, immunophilin). This uncovers nuclear localisation signal motifs in the hinge area of the receptor and translocation of the receptor to the nucleus is facilitated by proteins of the importin family. Critical to the outcome of the genomic effect of the GR is the recognition or highly specific DNA sites, the response elements (GRE). These areas promote stable binding to facilitate recruitment of co-regulatory proteins. Binding to the GRE causes allosteric changes promoting a protein surface that attracts co-regulatory proteins. The nucleotide sequence of the GRE determines the magnitude of the outcome. Variations in GRE binding affinities for the GR and for affinities for binding adjacent co-regulator sites results in variability in outcome. Both positive GREs and negative GREs have been identified with nGRE causing repression rather than activation. Co-regulator proteins are enzymically active proteins that once recruited alter the chromatin environment thereby facilitating or inhibiting assembly of the basal transcription machinery and the initiation of transcription by RNA polymerase II.

The transcriptional outcome is also dependent on the structure of the promoter region of the target gene and can result in activation (transactivation) or repression (transrepression) of the gene. Transactivation is triggered by recruitment of other transcription proteins such as activator protein 1 and nuclear factor-κB while transrepression occurs due to GR inhibition of transcriptional factors.

In summary:
Glucocorticoid binding to the GR results in conformational change to the receptor and nuclear translocation, DNA binding, co-regulator recruitment and chromatic re-organisation. There is considerable variation in outcome possible with glucocorticoid therapy based on

1. Non-genomic effects
2. Bioavailability of the drug (ligand) to bind the GR
3. The affinity for GR binding
4. The agonist potential of the drug (ability to induce receptor activation on binding)
5. Transcriptional potential of the GR (isoform expression)
6. Cell and tissue variability in receptor expression and target genes and cytokine influences on expression.
7. Affinity of GR for the GRE
8. Nucleotide sequence of the GRE
9. Binding affinity of GRE to co-regulatory sites
10. Transactivation versus transrepression

What do glucocorticoids do?
1. **Physiological effects.**

Restoring homeostasis involves intimate neuro-endocrine interactions. Cytokines and inflammatory mediators activate peripheral pain receptors whose axons project to the dorsal horn and synapse with the lemniscal tract, which in turn carries pain signals to the thalamus and the somatosensory cortex. Activation of this nociceptive pathway ultimately stimulates hypothalamic–pituitary–adrenal activity. Glucocorticoids inhibit the synthesis of cytokines and inflammatory mediators, thus forming a negative feedback loop. Cytokines can also act directly on the brain to activate the hypothalamic–pituitary–adrenal axis. Dysregulation of this neuroendocrine loop by hyperactivity (Cushing’s disease, chronic pain, stress) results in immunosuppression and hypoactivity (Addison’s) require exogenous glucocorticoids when infected to prevent excessive cytokine triggered tissue injury.
Table 1. **Tissue specific GC effects**

<table>
<thead>
<tr>
<th>System</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Nervous System</td>
<td>Euphoria and behavioural changes</td>
</tr>
<tr>
<td></td>
<td>Maintenance of alpha rhythm</td>
</tr>
<tr>
<td></td>
<td>Lower Seizure Threshold</td>
</tr>
<tr>
<td>Autonomic Nervous System</td>
<td>Required for normal sensitivity of adrenergic receptors</td>
</tr>
<tr>
<td>Gastrointestinal Tract</td>
<td>Decreased calcium and iron absorption</td>
</tr>
<tr>
<td></td>
<td>Facilitation of fat absorption</td>
</tr>
<tr>
<td></td>
<td>Increased acid, pepsin, and trypsin</td>
</tr>
<tr>
<td></td>
<td>Structural alteration of mucin</td>
</tr>
<tr>
<td></td>
<td>Increase insulin tolerance</td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>Weakness (excess and deficiency)</td>
</tr>
<tr>
<td></td>
<td>Muscle atrophy (chronic excess)</td>
</tr>
<tr>
<td>Skin</td>
<td>Atrophy and thinning (chronic excess)</td>
</tr>
<tr>
<td></td>
<td>Calcinosis Cutis</td>
</tr>
<tr>
<td>Hematopoietic system</td>
<td>Involution of lymphoid tissue (species dependent)</td>
</tr>
<tr>
<td></td>
<td>Decrease in peripheral lymphocytes, monocytes, eosinophils</td>
</tr>
<tr>
<td></td>
<td>Increase in peripheral neutrophils, platelets, RBCs</td>
</tr>
<tr>
<td></td>
<td>Decreased Clotting Time</td>
</tr>
<tr>
<td></td>
<td>Decreased phagocyte competence</td>
</tr>
<tr>
<td>Cardiovascular system</td>
<td>Positive inotrophic effect</td>
</tr>
<tr>
<td></td>
<td>Increased blood pressure (increased blood volume)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>Increased reabsorption of water, sodium, chloride</td>
</tr>
<tr>
<td></td>
<td>Increased excretion of potassium, calcium</td>
</tr>
<tr>
<td></td>
<td>Increased extracellular fluid</td>
</tr>
<tr>
<td>Liver</td>
<td>Increased gluconeogenesis</td>
</tr>
<tr>
<td></td>
<td>Increase lipid storage</td>
</tr>
<tr>
<td>Bone</td>
<td>Inhibition of collagen synthesis by fibroblasts</td>
</tr>
<tr>
<td></td>
<td>Acceleration of Bone resorption</td>
</tr>
<tr>
<td></td>
<td>Antagonism of Vitamin D</td>
</tr>
<tr>
<td>Cells</td>
<td>&quot;Stabilization&quot; of liposomal membranes</td>
</tr>
<tr>
<td></td>
<td>Inhibition of macrophage response to migration inhibition factor</td>
</tr>
<tr>
<td></td>
<td>Lymphocyte sensitisation blocked</td>
</tr>
<tr>
<td></td>
<td>Cellular response to inflammatory mediators blocked</td>
</tr>
<tr>
<td></td>
<td>Inhibition of fibroblast proliferation</td>
</tr>
<tr>
<td>Reproductive Tract</td>
<td>Parturition induced during the latter part of pregnancy in ruminants and</td>
</tr>
<tr>
<td></td>
<td>horses</td>
</tr>
<tr>
<td></td>
<td>Less reliable in dogs and cats</td>
</tr>
<tr>
<td></td>
<td>Teratogenesis during early pregnancy.</td>
</tr>
</tbody>
</table>


2. **Anti-inflammatory effects.**

Inflammation occurs following any irritant, traumatic or infectious insult. Microbes that breach the epithelial barrier activate innate immunological sentinels (complement, toll-like receptors) triggering the synthesis and release of inflammatory mediators with acute effects on the vasculature. Vasodilation, increased vascular permeability, extravasation of plasma (and humoral) proteins, and chemotaxis of leukocytes results in the grossly visible features of inflammation. As the innate and adaptative immune responses clear the infectious agent inflammation resolves. Endogenous glucocorticoids with HPA axis activation are essential to this resolution.

Exogenous glucocorticoids are indicated in sterile inflammation where homeostatic mechanisms fail and the inflammation (cytokine milieu) itself is responsible for the ongoing tissue injury.
GC inhibit prostaglandin synthesis by a number of independent pathways namely induction and activation of annexin 1 (lipocortin 1), induction of MAPK phosphatase 1 and repression of transcription of cyclooxygenase 2.

Annexin 1 interacts to inhibit cytosolic phospholipase 2 (cPLA2). cPLA2 when activated by inflammation moves from the cytosol to the perinuclear membrane and hydrolyses phospholipids releasing arachidonic acid that is then utilised to form inflammatory eicosanoids (prostaglandins, thromboxanes, prostacyclins, and leukotrienes).

MAPK cascades can be activated by viruses, UV light, inflammatory cytokines and bacteria. MAPK phosphorylation of intracellular kinases leads to binding to DNA sequences called activator protein 1 response elements and induce the transcription of numerous inflammatory and immune genes. Glucocorticoid-induced MAPK phosphatase 1 dephosphorylates and inactivates these kinases thus inhibiting transcription.

Nuclear factor κB (NF-κB) plays a major role in upregulating inflammation. It is a cytosolic protein sequestered by an inhibitory protein IκB. TNF-α, interleukin-1, microbial pathogens, viral infections, and other inflammatory signals trigger signaling cascades that activate IκB kinases. Phosphorylation of IκB (by IκB kinases) leads to its ubiquination and degradation by the proteasome, unmasking a nuclear localisation signal on NF-κB and leading to nuclear translocation. In the nucleus, NF-κB binds DNA sequences called NF-κB elements and stimulates he transcription of cytokines, chemokines, cell adhesion molecules, complement factors, and receptors for these molecules. NF-κB also induces the transcription of cyclooxygenase 2, an enzyme essential for prostaglandin production. Glucocorticoid- induced antagonism of NF-κB and repression of cyclooxygenase 2 are major mechanisms of GC anti-inflammatory actions. The many inflammatory cytokines inhibited by GCs are listed later under topical glucocorticoids (TG).

3. Anti-immunological effects
GC are potent immunomodulators/immunosuppressant agents depending on the dose and drug. They are reported to
- Induce redistribution of lymphocytes (species variable)
- Suppress cytokine and gene suppression
- Marked suppression of IL-1 and IL-6
- Retard neutrophil and macrophage chemotaxis
- Inhibit T-cell antigen recognition
- Inhibit T-cell proliferation
- Reduce Fc receptor mediated antibody dependent cytotoxicity and phagocytosis

Pharmacology
GC potency and duration of action is largely determined by the glucocorticoid base (unless the base is attached to an ester that prolongs its activity). In the case of orally administered drugs the ester is irrelevant as the esters are removed prior to absorption.

With injectable GCs the ester may control the amount of drug released into the circulatory system, which would also influence the magnitude of effect.
Table 2. Comparison of Glucocorticoid Bases.

<table>
<thead>
<tr>
<th>Base</th>
<th>Relative Potency</th>
<th>K/Na Effect</th>
<th>Equivalent Dose</th>
<th>Duration (HPA)</th>
<th>Structural Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short Acting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>1</td>
<td>++</td>
<td>20</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>Intermediate Acting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisolone</td>
<td>4.0</td>
<td>+</td>
<td>5</td>
<td>12 - 36</td>
<td>1=2</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>5.0</td>
<td>0</td>
<td>4</td>
<td>12 - 36</td>
<td>6-me; 1=2</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>5.0</td>
<td>0</td>
<td>4</td>
<td>12 - 36</td>
<td>9-F;16-OH;1=2</td>
</tr>
<tr>
<td><strong>Long Acting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betamethasone</td>
<td>25</td>
<td>0</td>
<td>0.8</td>
<td>&gt;48</td>
<td>9-F;16-bme;1=2</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>30</td>
<td>0</td>
<td>0.7</td>
<td>&gt;48</td>
<td>9-F;16-me;1=2</td>
</tr>
</tbody>
</table>

1. Glucocorticoid potency
2. Dose suggested is replacement therapy for a 20 kg. dog
3. Durations for other effects are likely to be different (see mechanism of action notes).


**Glucocorticoid Products**

Drug selection of a GC will vary depending on the intended duration of action and the potency. It is advisable to use the MINIMUM EFFECTIVE dose and for the shortest duration of activity. This implies that GC are being used AFTER a diagnosis has been reached and then only where additional intervention is necessary to control the inflammation. This means in cases where the clinician determines removal of the inciting cause of the inflammation does not achieve resolution of the inflammation by normal homeostatic mechanisms. In those cases the clinician will have an established therapeutic goal e.g. immunosuppression, anti-inflammatory and intended duration of action. This will allow for sensible therapeutic choices.

**Oral**

- The ester is irrelevant. All are separated from the base in the GI tract. The base drugs are well absorbed. Duration of action then is determined by the base. Dexamethasone is 7.5 X more potent than prednisolone and longer suppression of the HPA axis.

**IM, SQ, Intraliesional**

- Rapidly absorbed products can be used as substitutes for oral preparations. Their absorption and duration are roughly equivalent to the oral base products (and salts). Dexamethasone sodium phosphate would be an example of a rapidly absorbed injectable. These products are not suitable for intraliesional use.
- Slowly absorbed (Depot) products are designed to provide either low concentrations of glucocorticoids for extended periods of time or high concentrations in a local area. The poorly soluble phenylpropionate ester of dexamethasone in Dexaforté® would be an example. There is increased potential for local atrophogenic effects with depot injections and there are better administered IM than SQ.
- Intravenous use requires water soluble salts (prednisolone sodium succinate (Solu Delta Cortef®)). These reach sites of action 1/2 - 1 hour faster than oral but are otherwise similar in potency and are reserved for emergency therapy eg CNS trauma and shock.
Table 3. Available glucocorticoids

<table>
<thead>
<tr>
<th>Base</th>
<th>Oral</th>
<th>Intravenous Rapid IM, SC Absorption</th>
<th>Intralesional Slow IM, SC Absorption</th>
<th>Topical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betamethasone</td>
<td>Base</td>
<td>Na phosphate</td>
<td>Na phosphate + Acetate</td>
<td>Base Benzoate Dipropionate Valerate</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Base</td>
<td>Na phosphate</td>
<td>Acetate</td>
<td>Base</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>Base Cypionate</td>
<td>Na phosphate Na succinate</td>
<td>Acetate</td>
<td>Base Acetate</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>Base Na succinate</td>
<td>Acetate</td>
<td>Acetate</td>
<td>Acetate</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>Base</td>
<td>Na phosphate Na succinate</td>
<td>Acetate Tebutate Na phosphate + Acetate</td>
<td>Base Acetate Na succinate</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>Base Acetoniode Diacetate</td>
<td>Acetonide Diacetate Hexacetonide</td>
<td>Base Acetoniode</td>
<td></td>
</tr>
</tbody>
</table>


Anti-inflammatory Dosing

- Approximately 2-4 x replacement dose
- Usually 0.5 to 1 mg/kg prednisolone
- Alternate day therapy (or less) is recommended when treatment duration exceeds 4 weeks
- The HPA suppression potential of daily therapy is similar to the HPA suppression of eod therapy at 4 X the sid dose.
- 1/3 of dogs on long-term glucocorticoid therapy (> 6 months will have lower UTI)
- Targets for “safe” long term GC therapy (based on physiological cortisol levels in dogs) are 0.25 to 0.3mg/kg prednisolone twice weekly.
- Adjunctive, steroid sparing therapies are indicated in all dogs where long-term systemic GC therapy is needed at levels > 0.3mg/kg biw

Discontinuing therapy

- Cold turkey if glucocorticoid therapy of less than 2 to 4 weeks duration
- Taper off if Glucocorticoid therapy of greater than 2 weeks duration. Rate of taper should be proportional to duration of prior therapy. **The longer the original therapy, the slower the rate of dose reduction.**
- If on daily therapy reduce the alternate day dose by 25% per week to get to an eod dosage regime. Once on alternative day therapy treatment can be tapered rapidly.

Inhibit immunologic responses

Dosing

- Approximately 8- 16x replacement dose (daily)
- Usually initiate with 2-4 mg/kg prednisolone per day in two doses (2 mg/kg q12H)
Avoids relatively remote potential for acute adverse effects
- Possibly reduces initial efficacy (versus one single daily dose)
- Aggression, lethargy and behavioural changes possible on high doses.

Reducing dose rates
- Goal is to MINIMUM EFFECTIVE dose. Tapering should begin as soon as the disease is in clinical remission.
- With autoimmune diseases steroid sparing adjunctive therapy is mandatory where the duration of treatment is expected to be longer than 4 weeks.
- When tapering this should be done slowly eg 2-week minimum between dose changes, as disease flare may require returning to original remission doses.
- NOTE. Alternative day therapy does NOT avoid GC side effects when the doses used are supra-physiological. Alternative day therapy though will reduce the severity of the side effects compared with daily therapy.
- Daily therapy is however more POTENT for early suppression on the disease. In human medicine 90mg SID has the anti-immunological effect of 200mg eod.
- Take home message for immune suppression is daily to remission then taper and use adjunctive immunosuppressant therapies in all cases where the diagnosis dictates therapy for greater than 4 weeks.

Topical glucocorticoids (TG)
To reach the target cells the drug needs to pass through the barrier defense of the stratum corneum (SC). For a topically applied formulation containing a glucocorticoid the percutaneous absorption involves the following steps:

(a) Release from the formulation,
(b) Penetration into the skin’s outermost layer, the SC and permeation/diffusion through the SC,
(c) Partitioning from the SC into the viable epidermis and/or dermis,
(d) Within the viable epidermis/dermis, diffusion to reach the glucocorticoid receptor and binding.

The rate-limiting step is the penetration through the SC. This is affected by both the drug and the vehicle. Once through the SC the mechanism of action is the same as for systemic GCs. Modifying the chemistry of the drug alters the activity. Decreased mineralocorticoid and increased glucocorticoid activity (increased affinity for the GR) can be achieved by introduction of an additional double bond at C-1 and substitution at the C-16 position. The lipophilicity of the steroid and the duration of action are greatly increased by fluorination of the B ring at the C-9 and/or C-6 position. The lipophilicity and metabolic resistance of TG may also be increased by adding ester or acetal groups to the D-ring (e.g., betamethasone 17-valerate).

Methylprednisolone aceponate (Advantan®) and hydrocortisone aceponate (Cortavance®) and mometasone furoate (Elocon®) were developed as “soft” TG with good anti-inflammatory effects but less atrophogenic effects. The improved safety profile is associated with minimal suppression of IL-1 and IL-6 on dermal fibroblasts but marked suppression of epidermal IL-1. Di-esters are also very lipophilic so penetrate SC well and have high affinity to GR. They are de-esterified to form the active monoester in the epidermis and dermal penetration is low. These products are epidermally active with low dermal activity.

Topical glucocorticoids are often more potent than systemic steroids and have much greater systemic safety profile than oral or injectable GCs. TGs are indicated whenever there is regional disease and where owner’s compliance and patient’s temperament and coat type allow. They are particular useful for epidermal driven diseases eg canine atopic dermatitis.

In humans the atopic keratinocytes release pro-inflammatory cytokines IL-1, TNFα (tumour necrosis factor) and GM-CSF (granulocyte macrophage colony stimulating factor) at increased rates. IL-1 and TNFα induce a cellular signal cascade that results in release of the nuclear activation factor from its inhibitory complex and results in up regulation of numerous gene transcription factors. These are genes coding for pro-
inflammatory cytokines (IL-1, IL-2, IL-4, IL-6, IL-13, TNFα), chemotaxis proteins (e.g., GM-CSF), cellular adhesion molecules, i.e. intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1), AND also enzymes, i.e. phospholipase A2 (an enzyme responsible for the formation of prostaglandin and leukotrienes), inducible forms of cyclooxygenase (COX-2) and NO synthase (iNOS). All major players in inflammation. By inducing cellular adhesion molecules, pro-inflammatory cytokines and increase epidermal chemokine formation (CCL17, CCL22 RANTES, MCP-1 and CCL27) keratinocytes recruit and localize T-cells to the site of atopic inflammation AND ensure maximal activation of the cells.

By inhibiting these inflammatory cytokines, TGs interfere with the activation of a variety of immunologic cells. TGs inhibit the activation of epidermal dendritic cells that normally stimulate Th2 cells. Moreover, eosinophil and T cell apoptosis increases. TGs, as well as local anti-inflammatory and immunosuppressive effects have antiproliferative, and vasoconstrictive effects. The mechanism of action has been discussed previously. It is worth repeating though that it is likely that TOPICAL steroids reach high enough concentrations to exert both genomic and non-genomic effects while systemic steroids may struggle to achieve high enough concentrations in vivo to exert non-genomic effects.

Topical glucocorticoids are very effective at shutting down the epidermal events of inflammation. Not all steroids are created equal! The choice of steroid will depend on the severity of the inflammation and the type of lesion. The vehicle affects efficacy. For the same active ingredient ointments > creams > lotions > sprays as far as potency goes, due to occlusion and greater penetration. As always we need to consider the risk/benefit ratio.

Side effects of topical glucocorticoids.

**Epidermal thinning**

- Number of keratinocyte mitoses is diminished
- Stratum corneum thickness reduced
- Granular layer reduced or absent
- Melanocyte pigment production inhibited
- Reduced Keratin 5 and keratin 14 synthesis
- Inhibited K6/16 synthesis (poor wound healing)

**Dermal thinning**

Early Atrophy

- Dermal volume reduced – decreased water content, loss of glycosaminoglycans
- Collagen and elastic fibres unchanged

Late Atrophy (Continuation of the Atrophogenic Process)

- Dermal volume reduced
- Collagen and elastic fibres diminished and abnormally aggregated
- Hypoactive fibroblasts
- Dermal vessels fragile, due to loss of fibrous and ground substance support (capillary fragility)
- Follicular atrophy
- Follicular comedones

Topical steroids may be used once or twice daily in the acute situation. These may include Elocon® (mometasone), Diproson OV ® (betamethasone isopropionate), Cortavance ® (hydrocortisone aceponate) and Advantan ® (methyl prednisolone aceponate). The potency of steroids varies and compared to hydrocortisone the following potency is:

- 600 times more potent than hydrocortisone: Diproson OV
- 100 times more potent: Elocon, Advantan, Diproson cream or ointment (betamethasone valerate)
- 2-25 times more potent: Aristocort (triamicinolone acetonide)
Hydrocortisone is considered mild

NOTE: Potency and atrophogenic effects are NOT correlated due to variable affinities for glucocorticoid receptor binding and the complex mechanism of action. Mometasone is very potent but is less atrophogenic than hydrocortisone, which has low potency. Remember also that penetration may be affected by anatomical location. In people there is variability in systemic absorption with topically applied steroids with the genitals and eyelids (30%) and face (7%) and armpits (4%) having the greatest potential for local and systemic side effects. Areas of thin skin like the axillae, groin, umbilical fold and scrotum I would monitor closely for atrophic changes even with the di-ester steroids.

General rules and tips

Ointments are generally water in oil and are occlusive. Creams are usually oil in water. Lotions have more water than creams and often have alcohols to allow for quick drying. Gels are variable and may be occlusive or rapidly disappear depending on their chemical nature.

1. The vehicle is as important as the drug you are applying to the skin. Most of the non-shampoo topical treatments come as one or more of a lotion, spray, cream, gel or ointment. Moist, exudative skin is best treated with a cream or carbomer gel. Dry, scaling or lichenified skin is best treated with an ointment (hygroscopic properties and increased contact time). Lotions are good for hairy areas (far less messy) and are appropriate for areas where the skin is essentially normal –that is not lichenified or too dry. Sprays are good for relatively normal skin where treating larger areas but hair may significantly impede penetration and so are often best used on lightly haired skin OR used more generously on haired skin to increase penetration through to the epidermis.

2. Severe, focally inflamed and thickened skin can benefit from occlusion. Where a more rapid response is required occlusion will significantly increase absorption of the medication. E.g. Elocon ointment followed by gladwrap coverage, then Coflex bandaging - repeat twice daily for 20 to 30 minutes if needed

3. Occlusion can also be useful in some cases where there is a severe ‘contact’ distribution of inflammation (i.e. areas normally contacting the ground). Perform hydrotherapy (put in the bath for 5 to 10 minutes with just water), dry gently; apply Elocon cream or ointment depending on how dry the skin was. Alternatively wet an old T-shirt, wring so not dripping but still damp and place wet T-shirt on the dog and leave for 20 to 30 minutes after application of the steroid treatment. Occlusion and hydrotherapy hydrate the skin and increases penetration of the steroid and gives rapid relief.

Concluding thoughts.

Glucocorticoids are potent drugs and the therapeutic benefit needs to be balanced against physiological side effects. They are indicated after a diagnosis has been made and where glucocorticoids are indicated for the management of that diagnosis. Topical glucocorticoids should be used where disease, patient and owner factors allow. The minimum effective dose should be chosen for systemic GCs and potency and vehicle should be matched to the lesion type with TGs. Potentially treatment failure with GCs may occur in severe Th2 disease due to loss in genomic function. Better understanding of the non-genomic events may help with future treatment choices in these GC resistant cases. The current challenge in human medicine is to develop safer glucocorticoids. The greater understanding in intracellular events may lead to further development of selective agonists the selectively repress cytokine transcription without up regulating metabolic pathways (the selective glucocorticoid receptor agonists SEGRAs). It is likely that we will have safer tools in the future but for now it is worth remembering that all GCs are not created equal and choose appropriate dose, potency and type of GC based on the clinical diagnosis.
Further Reading

Azathioprine and chlorambucil: mechanism of action and use in dermatology

Dr. Fiona Bateman BVSc MACVSc

Introduction
Systemic immune moderators have been used in both human and veterinary dermatology for the treatment of a wide variety of immune mediated and allergic conditions. While their use is off label (even within the human field), a large amount of evidence attests to their safety and efficacy. Both azathioprine and chlorambucil have been used as single agent or, more commonly, in combination therapy of a wide range of dermatological conditions.

Azathioprine

History
Azathioprine was first developed as an anti-rejection drug for renal transplantation and was first used in combination with cortisone for this purpose in 1962. The discovery of azathioprine is one of the first examples of what is now termed ‘rational drug design’. George Hitchings and Gertrude Elion, researchers at Burroughs Wellcome Research Laboratories (now GlaxoSmithKline) were pioneers in drug development and attempted to identify cellular and molecular targets for which they then developed targeted drugs. One such drug was 6-MP (6-mercaptopurine), the precursor of azathioprine.

Hitchings and Elion hypothesized that the development of synthetic purine analogs may halt the growth of rapidly dividing cells. Thus they synthesized a variety of purine analogs, one of which was 6-MP. However, 6-MP proved to be rapidly metabolized in vivo, so in an attempt to increase its efficacy with the addition of an imidazole ring to the sulphur atom at position 6 (see Figure 1). The resulting compound, azathioprine, was more active and had a better safety profile than 6-MP. ¹

Pharmacology
Thiopurines are prodrugs that exert their cytotoxicity after they have been metabolized intracellularly. Despite over 50 years of use in human medicine, their exact mechanism of action remains incompletely characterized. The most biologically active end product of the thiopurines (including azathioprine) are 6-thioguanine nucleotides (6-TGNs). 6-TGNs inhibit de novo synthesis of purines and are incorporated into DNA as a false base, which triggers cell cycle arrest and apoptosis via the DNA mismatch repair mechanism. ²

Azathioprine is rapidly absorbed from the gastrointestinal tract following oral administration, with a half life of approximately 3 hours due to the rapid metabolism to 6-mercaptopurine (6-MP). The active

Figure 1.
Chemical composition of azathioprine, showing addition of imidazole ring to sulphur at position 6 of 6-MP

The long collaboration of Hitchings and Elion led to the development of some of the most successful drugs still used today, including allopurinol, pyramethamine (used to treat malaria), trimethoprim, acyclovir, and azidothymidine (AZT). Their discoveries were awarded (along with James Whyte Black) when in 1988, they received the Nobel Prize in Medicine.
metabolites have a much longer half life, which allows for once daily dosing. Azathioprine is extensively metabolized (Figure 2), with only 2% excreted unchanged in the urine.

Azathioprine is reduced through non-enzymatic degradation to 6-MP in vivo. This process occurs through nucleophilic attack by sulphahydryl compounds present in erythrocytes and body tissues. 6-MP is then metabolized by one of four pathways:

1. Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) – conversion to 6-thioinosine-5'-monophosphate (TIMP).
2. Thiopurine methyltransferase (TPMT) – catalyses S-methylation to 6-methyl mercaptopurine (inactive compound)
3. Xanthine oxidase (XO) – catalyses oxidation to 6-thiouric acid (inactive compound)
4. Aldehyde oxidase (AO) – conversion to 6-TGN hydroxylated metabolites (inactive compound)

The enzymatic competition for the 6-MP substrate is vigorous, with the effects of XO and AO activity leaving only 16% of the total dose of 6-MP for systemic distribution. Note this does not include TPMT activity, which will decrease the amount of 6-MP converted to TIMP further. The competing pathways are important in that blocking a metabolic pathway involved in the degradation of 6-MP (for example, through the use of a xanthane oxidase inhibitor such as allopurinol, or through low endogenous TPMT activity) can dramatically increase the available amount of 6-MP to be converted to active 6-TGNs, thereby drastically increasing the risk of severe adverse effects.

Once 6-MP is converted to TIMP, TIMP is then converted to 6-thioguanosine-5'-monophosphate (TGMP) in a 2 step process. TGMP is further metabolized through a series of reductases and kinases to form the 6-TGN metabolite, deoxy-6-thioguanosine-5'-triophosphate (dGS). dGS is then incorporated into DNA as a false base and triggers cell cycle arrest and apoptosis.

Figure 2. Metabolism of azathioprine to active 6-TGN metabolites.

**Mechanism of action**

The 6-TGN active metabolites disrupt the function of endogenous purines. While all cells are theoretically affected by this unstable base incorporated into DNA, RNA and proteins, lymphocytes are preferentially targeted by thiopurines. Lymphocytes rely on de novo synthesis of purines and lack a purine salvage pathway. Thus they are most affected by the action of azathioprine on purine synthesis and metabolism. Azathioprine has a wide range of short and long term effects on the immune system, including:

- reversible reduction of monocyte numbers in circulation and tissues
- impaired synthesis of gamma globulin (IgM, IgG) in patients with rheumatoid disorders
- long term immunosuppression decreases the number of cutaneous Langerhans cells
- impaired responses of helper T cell dependent B cells
- impaired function of T suppressor cells
- impaired T cell lymphocyte function and IL-2 production
interaction with Rac1, a triphosphate binding protein on T lymphocytes that mediates a costimulatory signal for T cell activation. Azathioprine inhibits Rac1, blocking the costimulatory signal and inducing Fas-associated apoptosis. In addition to the effects of 6-TGN metabolites, purine de novo synthesis is also inhibited by methyl-6-thioinosine monophosphate (Me-TIMP). Inhibition of de novo purine synthesis contributes to immunosuppression and blocks proliferation of various lymphocyte lines, thereby contributing to the cytotoxic action of azathioprine.

Azathioprine and TPMT activity

TMPT is the predominant inactivation pathway of thiopurines in haemopoetic cells. The end product of AZA degradation through the TMPT pathway is 6-methyl mercaptopurine (6-MMP), an inactive and nontoxic molecule. Erythrocyte levels of TPMT have been found to correlate well with levels in lymphocytes, platelets, kidney and liver cells in humans. TMPT activity is genetically controlled, with several polymorphisms identified in humans. In a recent study of over 3000 patients, approximately 80% had normal TPMT activity 9% had above normal enzymatic activity and 10% had low TPMT activity. Additionally, 0.45% of patients had no detectable TPMT activity. Low activity is associated with an increased risk of leukopenia, intermediate levels are associated with the development of late onset leukopenia and high TPMT activity results is less immunosuppression by azathioprine. Determination of pre-treatment TPMT level has been advocated in human medicine to detect patients at risk for early onset neutropenia (i.e. those with no or low TPMT activity). However the significance of TPMT activity in dogs and cats remains poorly characterized.

Studies of TPMT activity in the dog indicate that average levels of erythrocyte TPMT activity are similar to that in humans, but that marked variation exists in the distribution of TPMT activity when compared to the human studies. Interestingly, no dog was found with deficient TPMT activity (comparable to the human ‘low activity’ group) and that the 6 of 299 dogs in one study that experienced marked leukopenia associated with azathioprine use, all of these dogs had intermediate to high TPMT activity. This suggests that there exists a different mechanism by which azathioprine-induced myelotoxicity is induced in the canine population when compared with humans.

In cats, average levels of TPMT activity were significantly lower than both humans and dogs, and cats also displayed large individual variations in the level of TPMT activity. This is consistent with the high level of myelosuppression and leukopenia seen when azathioprine is administered to cats, and further supports the recommendation that azathioprine not be used in this species.

Studies of azathioprine use in the horse are limited, but TPMT activity is reported to be lower than both dogs and cats. Interestingly, marked myelosuppression (which would be expected to be marked given the low TPMT activity) is infrequently seen in the horse. This may indicate that erythrocyte TPMT activity may not correlate to TPMT activity in other tissues (particularly the liver) or that other degradation pathways, such as XO or AO may be more important for the metabolism of 6-Mp to inactive compounds in this species.

Indications in dermatology

Dermatologic use of azathioprine remains off label in both human and veterinary literature. Azathioprine has been used in dermatological conditions for over 50 years, and its use is supported by numerous studies, case reports and expert opinion. However, by the strictest evidence-based medicine standards, the support for its use is not as strong as for a variety of newer medications, such as cyclosporine.
Table 1.  Selected dermatologic diseases where azathioprine has shown to be of benefit

<table>
<thead>
<tr>
<th>Immunobullous disease</th>
<th>Photodermatitis</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bullous pemphigoid</td>
<td>Actinic reticuloid</td>
<td>Erythema multifforme</td>
</tr>
<tr>
<td>Pemphigoid</td>
<td>Chronic actinic dermatitis</td>
<td>Cutaneous lupus erythematosus</td>
</tr>
<tr>
<td>Cicatricial pemphigoid</td>
<td></td>
<td>Lichen planus</td>
</tr>
<tr>
<td>Juvenile pemphigus</td>
<td></td>
<td>Cutaneous vasculitis</td>
</tr>
<tr>
<td>Pemphigus foliaceus</td>
<td></td>
<td>Graft-versus-host disease</td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraneoplastic pemphigus</td>
<td></td>
<td></td>
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<tr>
<td>Pemphigus erythematosus</td>
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<table>
<thead>
<tr>
<th>Eczematous diseases</th>
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<tbody>
<tr>
<td>Psoriasis</td>
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<tr>
<td>Atopic dermatitis</td>
</tr>
</tbody>
</table>

Adapted from Patel, et. al.

Table 2.  Selected dermatologic diseases where azathioprine may be of benefit

<table>
<thead>
<tr>
<th>Pemphigus foliaceus</th>
<th>Lupoid onychitis ** 23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemphigus vulgaris</td>
<td>Uveodermatologic syndrome</td>
</tr>
<tr>
<td>Superficial pemphigus complex</td>
<td>Epidermolysis bullosa acquista 24</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>Perianal fistula **</td>
</tr>
<tr>
<td>Vesicular cutaneous lupus erythematosus** 25</td>
<td>Erythema multifforme 26</td>
</tr>
<tr>
<td>Cutaneous reactive histiocytosis ** 27</td>
<td>? Atopic dermatitis** 28</td>
</tr>
<tr>
<td>Idiopathic sterile granuloma and pyogranuloma</td>
<td></td>
</tr>
</tbody>
</table>

** indicates that other forms of drug therapy are the gold standard for treatment of this condition, but azathioprine may be of benefit in refractory cases.

Adverse effects

Haematologic

Cytopenias and severe bone marrow suppression have been reported in both the human and veterinary literature, and may occur weeks to months after initiating treatment. Humans may suffer from bone marrow suppression years after initiating therapy, as steady state levels of azathioprine in the blood may take months to years to achieve. While neutropenia is the most common cytopenia noted, anaemia and thrombocytopenia may also occur.\(^2\) TPMT genotype testing is advocated in human medicine to identify those individuals at risk for developing acute or delayed onset neutropenia. While TPMT activity has been measured in dogs, cats and horses, its relevance to the development of adverse effects is unknown.

While pretreatment TPMT activity is a useful indicator of susceptible individuals in humans, continuous hematological monitoring is still mandatory in humans and animals. Leukopenia induced by azathioprine, while potentially life threatening, is usually reversible with discontinuation of treatment or a dose reduction.

Gastrointestinal

Nausea and vomiting, though widely reported in the human literature, are uncommonly encountered in veterinary medicine. Symptoms usually occur in the first few weeks of therapy and may self resolve. Administration of azathioprine with food or in a divided dose may help to reduce the incidence of gastrointestinal upset.\(^2\)

Hepatotoxicity is the second most common gastrointestinal side effect and is independent of TPMT activity.\(^3\) In most cases, hepatotoxicity is an unpredictable side effect and the mechanism of hepatocyte injury is poorly characterized. In dogs, elevation of alkaline phosphatase, alanine aminotransferase and bilirubin may be transient, in other cases they may be persistent and progressive leading to hepatic failure and death. In a small pilot study using azathioprine for the treatment of canine atopic dermatitis, serum levels of alanine aminotransferase and alkaline phosphatase rose in 83% of dogs by the second week of daily treatment, and clinical signs of hepatitis were reported in 3 of 12 dogs (25%) necessitating the need for removal from the study.\(^28\)
Pancreatitis has been associated with azathioprine use in both the human and veterinary literature. In humans, pancreatitis mostly occurs in patients with concurrent gastrointestinal disorders. In dogs, azathioprine is commonly used in combination therapy (usually with corticosteroids) so a direct causal relationship may be difficult to determine.

**Opportunistic infections**

Long term immunosuppression has been associated with an increased risk of the development of opportunistic infections. This may occur even in the absence of leukopenia and herpes simplex, herpes zoster and verrucae have been reported at a higher incidence in humans receiving combination azathioprine and cortisone therapy.

Opportunistic infections have been reported in dogs, but the overall prevalence of opportunistic infections in animals on immunosuppressive doses is low.

**Carcinogenesis**

Controversy exists as to the potential link between long term immunosuppressive therapy with azathioprine and increased risk of malignancy. While some authors suggest that there is currently no evidence that thiopurine therapy is associated with an increased risk of malignancy, other studies indicate that dermatology patients on long-term azathioprine therapy may be at risk of developing aggressive squamous cell carcinoma, particular where the patient has had excessive exposure to UV light. Finally, yet further studies have indicated that while an increase risk of squamous cell carcinoma is present in renal transplant patients, it appears to be independent of the drug used. In this study, either long term cyclosporine and azathioprine with or without corticosteroids showed no difference sin cancer risk between the groups.

The method by which carcinogenesis is proposed to occur (if at all) is due to the incorporation of 6-TGN metabolites into DNA. Once this process occurs, the DNA becomes prone to oxidation due to the high reactivity of the thiobase. Exposure to UVA light destabilises the double helix and sensitises the cell to the mutagenic effect of UV light, which is believed to be one of the causes of azathioprine-related malignancies, in particular squamous cell carcinoma. Additionally, azathioprine causes inactivation of the mismatch repair system in myeloid precursor cells which can lead to development of drug-resistant cells.

**Hypersensitivity reactions**

Rare reports exist of azathioprine hypersensitivity syndrome in humans. Clinical signs may include hypotension, shock, urticarial or vasculitic eruption, fever and rhabdomyolysis. To the authors knowledge, similar reactions have not been reported in the veterinary literature.

**Miscellaneous**

When administered with isotretinoin, azathioprine has been reported to induce curling of the hair in humans.

**Contraindications and drug interactions**

Azathioprine should not be used in patients with a known hypersensitivity to the drug. In addition dosage adjustments may need to be made in cases of renal or hepatic insufficiency. As the safety margin for use in cats is extremely low, it is not recommended for use in this species. Use in pregnant animals should be with care as azathioprine is both mutagenic and teratogenic in lab animals, though no clear-cut relationship between the drug and sporadic reports of human congenital anomalies has been accepted. Use should only be considered where the benefits clearly outweigh the risk and clients should be adequately counselled. There is no evidence that azathioprine produces gonadotoxicity or infertility in humans.

Xanthine oxidase inhibitors such as allopurinol, should not be used in combination with azathioprine where possible. Allopurinol inhibits the metabolism of azathioprine to inactive metabolites, thereby increase the amount of azathioprine available for metabolism to 6-TGNs. If allopurinol must be used, the dose of azathioprine should be reduced by at least 2/3rds, however the risk of myelotoxicity remains. Angiotensin-converting enzyme inhibitors (ACEIs) have been shown to potentiate the effects of azathioprine in humans. Additionally, trimethoprim-sulfamethoxazole is an antimetabolite that has a synergistic effect in inhibiting bone marrow proliferation. However, the clinical significance of the interaction of these drugs with azathioprine has not been seen in the non-renal transplant setting. Sulfasalazine is an inhibitor of TPMT activity and may potentiate azathioprine toxicity. Current therapeutic guidelines advise against concurrent use of these medications with azathioprine where possible.

Warfarin resistance has been reported in the humans, however azathioprine toxicity is not enhanced by warfarin, rather the effects of warfarin are reduced. While this is a notable drug interaction in the human
literature, warfarin is rarely used therapeutically in animals and may be less of a concern in veterinary medicine.

**Dosage and monitoring**

Azathioprine (Imuran®, GlaxoSmithKline) is available in 25mg and 50mg tablets and as a sodium salt for injection (50mg vial). The recommended dose for azathioprine in dermatology in humans and dogs is reported to be 1-2.2 mg/kg orally daily, reducing to every other day administration after 1-2 weeks of daily therapy. However, many clinicians advocate the use of a body surface area derived dose rate (50mg/m²) in animals over 20kg.

In humans, a new dosing system (Table 3) has been proposed based on pretreatment TPMT activity. While TPMT is a useful indicator of potential risk groups for adverse haematologic effects, the role of TPMT activity in dogs, cats and horses remains unclear so dosing based on TPMT activity in these species is not recommended.

**Table 3. Proposed new dosing schedule (human) – adapted from Patel, et al.**

<table>
<thead>
<tr>
<th>Patient group</th>
<th>TPMT activity (U/ml rbcs)</th>
<th>Suggested max. dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No activity (homozygous mutation)</td>
<td>&lt; 5</td>
<td>Recommend not using</td>
</tr>
<tr>
<td>Low activity (heterozygous)</td>
<td>5-13.7</td>
<td>1</td>
</tr>
<tr>
<td>Normal activity (homozygous wild type)</td>
<td>13.8-19.5</td>
<td>2.5</td>
</tr>
<tr>
<td>High activity (high homozygous)</td>
<td>&gt; 19.5</td>
<td>3</td>
</tr>
</tbody>
</table>

Therapeutic response to azathioprine occurs in 6-8 weeks. If a response is not seen, then the azathioprine dose may be increased by 0.5 mg/kg at 4 week intervals with reference to white cell counts and clinical response, but a total dose of 3mg/kg should not be exceeded. If there is no response to treatment after 12-16 weeks, azathioprine should be discontinued.

No formal guidelines exist for hematological or biochemical monitoring of azathioprine in dermatology patients in the human or veterinary fields. Baseline complete blood count (CBC) and serum biochemistry panels should be run prior to the initiation of therapy. The author then repeats the CBC at weekly intervals for the first 4 weeks of therapy, then fortnightly to 8 weeks of therapy, monthly to 16 weeks then every 3 months thereafter. Biochemical analysis (with particular reference to liver function testing) is repeated 2 weeks after initiation of therapy, and then if any evidence of gastrointestinal upset, inappetence, fever, malaise or icterus is present. Ideally, urine culture and sensitivity should be performed prior to the initiation of therapy and then every 3 months, due to long term immunosuppression and the risk of occult urinary tract infections (note that this risk is not specific to azathioprine).

**Chlorambucil**

**History**

Chlorambucil is a potent alkylating agent used in a range of neoplastic and non-neoplastic dermatological conditions. The development of the alkylating agents, including chlorambucil, have revolutionised cancer chemotherapy. Alkylating agents are derivatives of mustard gas (nitrogen mustards), which were extensively used as chemical warfare agents in both World War I and II. Top secret studies carried out in the early to mid 1940s revealed that when these agents were administered systemically they were highly cytotoxic, with the degree of cytotoxicity positively correlated with the proliferative capacity of the cells – thus nitrogen mustards and their derivatives preferentially killed highly proliferative organs such as the gastrointestinal tract, bone marrow and lymphoid tissues.

The first clinical report of nitrogen mustards using in cancer chemotherapy was published by Goodman et al in 1946, when 67 patients with Hodgkin’s lymphoma, leukaemia and lymphosarcoma were treated with a nitrogen mustard derivative. Significant improvement was seen in a number of patients, but the margin of safety of the drug in these individuals was narrow.

Chlorambucil was developed in 1953 by Everett et al, with the addition of an aryl group to the nitrogen mustard molecule known as bis-(2-chloroethyl)amine, Addition of other active moieties onto this base molecule led to the development of a number of more targeted drugs such as melphalan and cyclophosphamide.

**Pharmacology and mechanism of action**

Alkylating agents exert their effect directly on DNA, RNA and proteins, usually by non specific means. The chlorine groups on the nitrogen mustard facilitate nucleophilic attack of nitrogen to form an imminium ion (R₂N). This highly reactive ion undergoes alkylation at N7 of guanine to form a
monoalkylated product on the DNA strand. Repetition of this cycle causes cross-linking of DNA. In the case of chlorambucil, two complementary strands of DNA are cross-linked. Cross-linking of DNA prevents separation of DNA strands for transcription and subsequent failure of transcription leads to apoptosis. Chlorambucil can also covalently bond to RNA and proteins through a similar mechanism.

Figure 4. Chemical composition of chlorambucil

\[
\text{HO} \quad \text{O} \quad \text{Cl} \\
\text{Cl} \quad \text{N} \quad \text{Cl}
\]

Chlorambucil is considered cell cycle non-specific. Following oral administration, chlorambucil is rapidly and nearly completely absorbed from the gastrointestinal tract. It is highly protein bound in plasma. The major route of metabolism is spontaneous hydrolysis, though chlorambucil is also metabolized in the liver to form phenylacetic acid mustard (active compound). Phenylacetic acid mustard is further metabolized to inactive products which are excreted in the urine and faeces.

**Indications in Dermatology**

**Human**

<table>
<thead>
<tr>
<th>Table 1. Selected dermatologic diseases where chlorambucil has shown to be of benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neoplastic disease</strong></td>
</tr>
<tr>
<td>Cutaneous T cell lymphoma</td>
</tr>
<tr>
<td>Sézary syndrome</td>
</tr>
<tr>
<td>Cutaneous B cell lymphoma</td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
</tr>
<tr>
<td>Pemphigus foliaceus</td>
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**Veterinary**

<table>
<thead>
<tr>
<th>Table 2. Selected dermatologic diseases where chlorambucil may be of benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mast cell tumour</strong></td>
</tr>
<tr>
<td>Feline eosinophilic granuloma complex</td>
</tr>
<tr>
<td>Pemphigus foliaceus</td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
</tr>
<tr>
<td>Superficial pemphigus complex</td>
</tr>
<tr>
<td>Bullous pemphigoid</td>
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</tbody>
</table>

**Adverse effects**

**Haematologic**

Bone marrow toxicity is the most common side effect of chlorambucil therapy. It may be mild and transient, or severe and progressive. Myelosuppression is manifested by anaemia, leukopenia and thrombocytopenia. Leukocyte nadir may take 7-14 days, with recovery from 7-28 days. Severe pancytopenia may take months to years to achieve full recovery.

**Gastrointestinal**

Nausea and vomiting are frequent side effects of alkylating agent administration, including chlorambucil. Nausea has been reported within minutes of administration of the drug in humans, but may takes hours or days to become clinically apparent. In general, traditional antiemetics are poorly effective in controlling vomiting with these agents. Intractable nausea and/or vomiting may require hospitalization and symptomatic therapy. A dose reduction should be considered in severe cases.

Hepatotoxicity has been documented in humans and animals with alkylating agents, however chlorambucil has a higher safety margin than other alkylating agents (e.g. lomustine) thus fatal hepatotoxicity with this drug is rarely reported in the human literature and, to the authors’ knowledge, has not been reported in the veterinary literature.
Carcinogenesis
Due to their mutagenic properties, patients receiving alkylation therapy have been shown to have an increased risk in developing a second malignancy. Acute leukaemia is most frequently described as a second malignancy in humans, and usually develops within 1-4 years after drug exposure. The phenomenon has not yet been documented in veterinary literature, possible due to the short treatment lengths in these species.

Hypersensitivity reactions
Anaphylaxis, urticaria and drug eruptions have been reported with chlorambucil use in humans. Reactions to topically apply alkylating agents may also sensitise to systemically administered compounds.

Miscellaneous
Interstitial pneumonitis and pulmonary fibrosis have been reported in the human literature but not identified in veterinary medicine. Interestingly, a cumulative effect seems to be required as pulmonary fibrosis secondary to chlorambucil therapy has been noted after the discontinuation of therapy.

Alkylating agents have a significant toxic effect on reproductive tissue leading to ovarian atrophy and aspermia. As chlorambucil damages DNA at a fundamental level, it is also considered teratogenic.

Alopecia a delayed regrowth of the hair coat has been reported in dogs, with Poodles and Kerry Blue Terrier more likely to be affected than other breeds. As with azathioprine, the theoretical risk of opportunistic infections may be increased with long term immunosuppression, but little data exists in the human or veterinary literature on the prevalence of opportunistic infections associated with chlorambucil use.

Contraindications and drug interactions
Chlorambucil should not be used in patients with a known hypersensitivity to the drug. In addition dosage adjustments may need to be made in cases of renal or hepatic insufficiency. Use in pregnancy should be avoided unless the benefits clearly outweigh the risk.

The principle concern for development of myelosuppression is concurrent use of antineoplasics, immunosuppressants (e.g. azathioprine, corticosteroids, cyclophosphamide) and other bone marrow suppressive agents (e.g. chloramphenicol, flucytosine, amphotericin B, griseofulvin, colchicine).

Dosage and monitoring
Chlorambucil (Leukeran®, GlaxoSmithKline) is available in a 2mg tablet. Doses range from 0.1-0.2 mg/kg (dog and cat) administered orally every 24-48 hours. Concurrent use of corticosteroids may be required in the induction phase, once clinical response is seen then maintenance on every other day dosing of chlorambucil has been reported.

As with azathioprine, no formal guidelines exist for hematological or biochemical monitoring of chlorambucil in dermatology patients in exist. Baseline complete blood count (CBC) and serum biochemistry panels should be run prior to the initiation of therapy. The author then repeats the CBC at weekly intervals for the first 4 weeks of therapy, then fortnightly to 8 weeks of therapy, monthly to 16 weeks then every 3 months thereafter. Biochemical analysis is then only repeated if there is any evidence of gastrointestinal upset, inappetence, fever, malaise or icterus present. Ideally, urine culture and sensitivity should be performed prior to the initiation of therapy and then every 3 months.
References

Immunosuppressive therapy is a commonly undertaken therapy in the field of small animal internal medicine. Immune mediated haematological disorders including immune mediated haemolytic anaemia (IMHA) and immune mediated thrombocytopenia are commonly treated in canine medicine, with immune mediated neutropaenia seen less frequently. Musculoskeletal diseases requiring therapy include immune mediated polyarthritis, feline progressive polyarthritis and some cases of myositis, including eosinophilic myositis and dermatomyositis. Neurological disorders include granulomatous meningoencephalomyelitis and myasthenia gravis. Respiratory indications include allergic airway diseases, and in the gastrointestinal tract inflammatory bowel disease. More generalised disorders can include vasculitis, systemic lupus erythematosus or Sjogren’s syndrome. Internists will occasionally be called upon to treat immune mediated dermatological disorders (when there is no access to a dermatologist) such as pemphigus.

In many cases combination therapy will be used to treat such immune mediated diseases. In the majority of cases prednisone/prednisolone will be one of the drugs used, but other drugs used in combination may include azathioprine, cyclosporine, chlorambucil, vincristine and leflunamide – these drugs are used primarily to help increase the efficacy of therapy, and also for their steroid sparing effects. Drugs such as cyclophosphamide and danazol were used historically, but are currently rarely employed. In some diseases such as IMHA other therapy may be required to manage other disease complications, such as heparin to manage the risk of thromboembolic disease.

The presentation will cover basic pharmacology for some of the medications, and discussion of some of the protocols used by the author.

**Drug Pharmacology**

**Glucocorticoids - Prednisone/Prednisolone**

Glucocorticoids have both direct and indirect effects on the immune response. They have both anti-inflammatory and immunosuppressive effects, and ultimately both humoral and cell mediated arms of the immune response are affected. They inhibit early and late phases of inflammation, including oedema formation, leucocyte migration, phagocytosis, collagen deposition, and capillary and fibroblast proliferation. Glucocorticoids stabilise endothelial cell membranes, inhibiting production of local chemotactic factors, which reduce local infiltration of neutrophils, monocytes and lymphocytes. There is also reduced release of destructive proteolytic enzymes from tissues. Glucocorticoids inhibit the release of arachadonic acid from membrane phospholipids, thereby reducing the synthesis of prostaglandins, thromboxanes and leucotrienes, which are all mediators of inflammation. Glucocorticoids also inhibit the release of tumour necrosis factor and interleukin-2 from activated macrophages, and platelet activating factor from leucocytes and mast cells. They also induce the redistribution of monocytes and lymphocytes from the peripheral circulation to the lymphatics and bone marrow, especially T-cells. Glucocorticoids may also have some potential genomic effects via effects on specific DNA sequences called glucocorticoid responsive elements, which can then alter transcription of nearby genes, either positively or negatively. Glucocorticoids have minimal effects on plasma immunoglobulin concentrations. Glucocorticoids in addition may reduce macrophage Fc receptor expression, suppress neutrophil and monocyte bactericidal function, reduce antigen presentation to T helper cells, reduce effector cell function such as natural killer cells, and inhibit amplification pathways of the complement cascade.

The chronic effects of glucocorticoid usage can be significant challenge for pet owners, with signs of iatrogenic hyperadrenocorticism. Side effects including polydipsia/polyuria, polyphagia and panting can result in significant affects for owners. Other problematic side effects can include calcinosis cutis, muscle atrophy and weakness, increased risk of infection, and in some cases diabetes mellitus. In some situations more local glucocorticoid therapy has been used to try and reduce the degree of systemic side effects from glucocorticoids. These include budesonide and fluticasone. Budesonide has been used inhalationally, but can be used orally to help manage inflammatory bowel disease. It is a non-halogenated glucocorticoid that is absorbed from the gastrointestinal tract, but undergoes extensive (90%) hepatic metabolism – the metabolites have minimal activity and are renally excreted. After absorption the budesonide is reversibly converted to lipophilic esters within cells, and gradual hydrolysis of the esters prolongs its local effect. Its efficacy for...
inflammatory bowel disease is variable. Fluticasone propionate is an androstane glucocorticoid used inhalationally to treat inflammatory airway disease. Both drugs result in some suppression in the hypothalamic-pituitary-adrenal axis with extended usage, but less than for oral prednisone.

Antimetabolites
These drugs alter cell function via interference with enzyme function, or promoting the synthesis of an altered molecule that fails to function within the cell normally. Drugs that fall into this category include methotrexate (an anti-folate mechanism), cytosine arabinoside ([a pyrimidine analogue) and azathioprine (a purine analogue). These drugs are S phase cell cycle specific.

Cytosine arabinoside is transported into cells and metabolised to 5'-triphosphate ara C which inhibits DNA polymerase and is then incorporated into DNA preventing templating of DNA and inhibiting repair. The drug is used in some chemotherapy protocols (lymphoid). It has also been more recently used in combination with prednisolone for the treatment of meningoencephalomyelitis of unknown aetiology, at a dose rate of 50mg/m² every 12 hours for 48 hours, repeated every three weeks. Myelosuppression is a potential side effect, along with gastrointestinal upset.

Azathioprine is a commonly used immunosuppressive medication in dogs. It is metabolised to 6-mercaptopurine in the liver. Other ribonucleoside monophosphates are produced that accumulate in cells and have negative feedback on enzymes required for synthesis or purine nucleotides. The purine analogues also result in formation of non functional nucleic acid strands that prevent cellular proliferation, and immunosuppression results from reduced DNA and RNA synthesis, and inhibition of co-enzyme formation and mitosis. Azathioprine is considered to have more effect on humoral immunity than cell mediated immunity. Effects on T-cells include inhibition of inflammatory gene expression, induction of apoptosis following activation, and suppression of conjugate formation with antigen presenting cells. There is reduced release of proinflammatory cytokines released by macrophages and monocytes.

Azathioprine is commonly administered in combination with prednisolone for the treatment of many immune mediated diseases including immune mediated haemolytic anaemia, immune mediated thrombocytopenia, and immune mediated polyarthritis. Dose rates include 50mg/m² or 1-2 mg/kg every 24-48 hours. Azathioprine can cause profound myelosuppression in cats, with a dose rate of 0.3 mg/kg every 48 hours noted, but its use is not recommended because of the challenge of accurately dosing cats. Side effects can include bone marrow suppression (anaemia, thrombocytopenia, neutropaenia), and possibly pancreatitis and hepatotoxicity.

Mitotic Inhibitors
Vincristine is a vinca alkaloid extracted from the periwinkle plant. It binds to tubulin, a microtubular protein within cells, acting as a spindle poison. The spindles then cannot act in mitosis, arresting the cell cycle in metaphase. Vinca alkaloids also lead to breakdown of preformed microtubules which function in maintenance of cellular structure and provide a conduit for secretions and neurotransmitters along axons.

Vincristine is primarily used as a chemotherapeutic medication, especially lymphoid neoplasms, and canine transmissible venereal tumour. The drug has also been used as part of the therapy for immune mediated thrombocytopenia, where it increases the release of platelets from megakaryocytes. It can be administered as a bolus, or it can be administered with a transfusion of platelet rich plasma (loading platelets). Side effects of vincristine include tissue necrosis if extravasated, myelosuppression, gastrointestinal upset and neurotoxicity, associated with neuronal degeneration with axonal swelling and secondary demyelination of peripheral nerves.

Alkylating Agents
Chlorambucil is a derivative of nitrogen mustard, and is the slowest acting and least toxic of the alkylating agents used. The alkylating agents react covalently with DNA, resulting in breaks in the molecule and cross linking of the twin strands. The net result is interference with DNA replication and RNA transcription, inhibiting protein synthesis in resting cells, prevention of mitosis and death of dividing cells. Chlorambucil has a high oral bioavailability if administered without food, and is metabolised in the liver to phenylacetic acid mustard.

Chlorambucil is used as an antineoplastic for chronic lymphocytic leukaemia and lower grade lymphoma (such as low grade alimentary lymphoma in cats). It has also been used in combination with other drugs such as prednisolone to treat some dermatological diseases, and immune mediated disease in cats (immune
mediated haemolytic anaemia, immune mediated thrombocytopenia, inflammatory bowel disease). Side effects include myelosuppression, and occasionally gastrointestinal upset.

**Calcineurin Inhibitor: Cyclosporine**

Cyclosporine binds in the cytosol of lymphocytes to cyclophilins. The cyclosporine-cyclophilin complexes associate with calcineurin-caldesmon complexes, which impede calcium dependant signal transduction. The calcineurin-caldesmon complex is a calcium dependant serine threonine phosphatase that results in dephosphorylation of regulatory proteins. The dephosphorylated proteins move to the nucleus and act as subunits of certain transcription factors, one of which (NF-AT) promotes transcription of the Interleukin-2 (IL-2) gene. Cyclosporine therefore suppresses transcription of key cytokines involved in innate and adaptive arms of the immune response including IL-2 which is a T-cell growth factor, and interferon-α, a monocyte-macrophage activation factor. Cyclosporine also inhibits proliferation of canine keratinocytes and reduced their synthesis of prostaglandin E2. Cyclosporine can also stimulate mammalian cells to secrete transforming growth factor-β, which is a potent inhibitor of IL-2 stimulated T-cell proliferation.

Cyclosporine usage has increased in recent years in veterinary medicine in many situations where immune suppression has been required. It has been used in both canine and feline transplant patients, and immune mediated diseases such as immune mediated haemolytic anaemia, immune mediated thrombocytopenia, immune mediated skin diseases, pure red cell aplasia, refractory cases of inflammatory bowel disease, perianal fistulae, and keratoconjunctivitis sicca (topically).

There are intravenous preparations of cyclosporine available that has been solubilised and is diluted and administered as an intravenous infusion diluted in 0.9% sodium chloride. There are oral forms available of the drugs that are a microemulsion formulation (Neoral, Atopica) that have replaced the older oral formulations such as Sandimmune (olive oil base) that are more erratic with absorption. Cyclosporine undergoes hepatic metabolism and biliary excretion, with minimal renal excretion. The dosage of the microemulsion form is 5-10 mg/kg/day divided in dogs, and 1-5 mg/kg/day divided in cats. Therapeutic drug monitoring is an option, but is considered somewhat controversial because of the more consistent oral absorption of the newer drug formulations, and also the apparent lack of correlation of drug levels and clinical efficacy. Care should be taken in dogs affected by the mdr-1 defect as cyclosporine is a p-glycoprotein substrate. There have been recommendations in the past to co-administer cyclosporine with ketoconazole to inhibit the cytochrome p-450 isoenzyme involved with the metabolism of cyclosporine, thereby potentially reducing the dosage of cyclosporine required. In this situation therapeutic monitoring would be recommended.

Side effects of cyclosporine have been recognised including vomiting, diarrhoea, inappetance, weight loss, gingival hyperplasia, papillomatosis and gingival hyperplasia. Hepatotoxicity and nephrotoxicity are rarely recognised in dogs at therapeutic dosages. Long term therapy may increase the probability of neoplasia, especially lymphoma, especially if there is concurrent prednisone therapy. There is an increased risk of opportunistic bacterial and fungal infections in dogs and cats. In cats recrudescence of toxoplasmosis has also been reported. There was a recent abstract at ACVIM that reported a potential risk of increased procoagulant activity of platelets in animals being treated with cyclosporine.

**Leflunamide**

Leflunamide is a synthetic organic isoxazole metabolised by the intestinal mucosa to an active form. It inhibits de novo pyrimidine biosynthesis, reducing T and B cell proliferation. It may also have an antiproliferative effect on smooth muscle and fibroblast proliferation.

Leflunamide has been used to treat some more refractory immune mediated diseases in dogs, and has also been used in transplant patients. The suggested dose rates in dogs are 4 mg/kg over 24 hours, and 2 mg/kg once or twice a week in cats. Side effects reported include leucopaenia, thrombocytopenia and gastric ulceration.

**Human Intravenous Immunoglobulin**

Human intravenous immunoglobulin (hIVIG) is a purified IgG derived from the plasma of healthy humans, and it is used to treat a number of primary and secondary immunodeficiency syndromes and some immune mediated disorders including chronic inflammatory demyelinating polyneuropathy, immune mediated haemolytic anaemia, immune mediated thrombocytopenia, immune mediated neutropaenia, pure red cell aplasia, acute myasthenia gravis, Guillain-Barre syndrome and dermatomyositis. Some of the suggestive mechanisms of action include blocking of Fcγ receptors on monocytes and macrophages, saturation of Fe
receptors on endothelial cells, neutralisation of autoantibodies by idiotypic antibodies in hIVIG, and inhibition of interaction of autoreactive T-cells with antigen presenting cells.\(^3\)

There have been a number of cases of the use of hIVIG in dogs, including for non regenerative anaemias, IMHA, IMT, Evan’s syndrome and a number of immune mediated skin diseases. There are some reports of success, whereas other studies did not show as positive a response.\(^3,9,10,11,12\)

The dose rates vary from 0.5 to 1.5 g/kg diluted in sterile 0.9% sodium chloride. Side effects reported include a mild, transient thrombocytopaenia, but repeated administration could increase the risk of hypersensitivity reactions.\(^3\) The high cost of the therapy can also be a limiting factor.

References

Open Forum: Immunosuppressive therapy – the dermatologist’s approach: formulation, protocols, weaning, monitoring, challenges

Chair: Mike Shipstone
Peter Hill, Rebecca Bassett, Linda Vogelnest, Graham Swinney

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Erythema multiforme and toxic epidermal necrolysis

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Erythema multiforme (EM) and Stevens-Johnson syndrome (SJS) are rare human and animal skin disorders with or without mucous membrane involvement. Toxic epidermal necrolysis (TEN) has been considered as a severe form of EM or SJS, but at the present time there are no generally accepted clinical and diagnostic criteria for the distinction between SJS and TEN. EM is further classified into two categories, EM minor or major, depending on the existence of mucocutaneous lesions.

Classification of EM/SJS/TEN in veterinary medicine

In an article in 1998, EM/SJS/TEN in dogs was classified through the adoption of a human international consensus clinical classification system. In this article, cases of EM/SJS/TEN were sub-classified into EM minor, EM major, SJS, SJS/TEN overlap syndrome and TEN based on the following signs: (1) flat or raised, focal or multi-focal, target or polycyclic lesions; (2) number of mucosal surfaces involved; (3) erythematous, pruritic, macular or patch eruption (% of body surface); and (4) epidermal detachment (% of body surface). According to the adoption of the human international classification system, canine EM cases are usually not associated with a history of drug exposure, while SJS, overlap syndrome and TEN are usually related to the administration of drugs.

In human medicine, EM minor, which exhibits as indurated erythema in the extremities, is the most common form of EM/SJS/TEN, whereas EM in dogs is recognised by dermatologists to be mainly EM major or SJS. Canine EM minor usually manifests as slight cutaneous changes, like peripherally raised focal erythema, without symptoms, that are often hidden by hair. Therefore many of these cases may have been overlooked by owners and veterinarians. That might be one of the reasons why the occurrence of EM minor in dogs is much less frequently diagnosed than that in humans.

Clinical signs of EM/SJS/TEN

There have been no reports of predilection based on gender, breed or age. Cutaneous presentation of EM presents as annular, erythematous macula, papules and plaques which become enlarged from the centre and often form a bizarre polycystic pattern. Target lesions become vesicular or bullous and then become necrotic, before finally forming ulcers. The lesions are often distributed around the oral mucous membrane, tongue, axilla, ventral abdomen, groin and central dorsal regions.

Histopathology of EM/SJS/TEN

The histopathology of EM/SJS/TEN in dogs includes interface dermatitis with marked apoptosis of keratinocytes at all levels of the epithelium and hair follicles. Mononuclear cell infiltration into the epidermis and/or mucous membrane epithelium and dermis were more intense in the SJS/TEN group than the EM minor and major groups. A large scale study implicated that histopathological examination should be restricted to confirmation of the diagnosis of diseases in the EM/TEN group, but not to subcategorise the different entities.

Pathogenesis of EM/SJS/TEN

In the only article that examined canine EM by immunohistochemistry, the expression of the infiltrates and keratinocytes was investigated. The intra-epithelial infiltrates were CD3⁺, CD8αβ⁺, and TCRαβ⁺ T cells. CD1⁺ and CD11c⁺ Langerhans cells were more numerous, whereas infiltrating cells in the dermis consisted of CD3⁺, CD8αβ⁺ and TCRαβ⁺ T cells. In dogs, it is believed that keratinocyte apoptosis is probably induced by signals from intraepithelial CD8⁺ T lymphocytes. Noli et al. implies that the cause of the massive epidermal cell necrosis in TEN is not immune mediated, but toxic, and was observed by the staining of apoptotic cells in EM and TEN cases.

A recent article regarding human SJS/TEN expressed that keratinocyte apoptosis was triggered by drug-specific cytotoxic T lymphocytes using perforin/granzyme B. Fas-mediated apoptosis may contribute to the extent of keratinocyte death, but it remains controversial whether the membrane-bound or the soluble form of Fas is responsible, and the cellular source from which it originates. Cytokines produced by T lymphocytes, macrophages, and possibly by keratinocytes themselves are thought to contribute to the pathogenesis of the SJS/TEN spectrum.
EM/SJS/TEN therapy

The most important factor in the therapy of EM/SJS/TEN is investigation and elimination of the triggering factors.\(^5,8\) When this is accomplished, the symptoms are usually resolved within 1–2 weeks.\(^8\) In idiopathic cases of EM/SJS/TEN, a large dose of glucocorticoids with azathioeprine and/or cyclosporine A may improve clinical signs. However, in human medicine, the use of glucocorticoids for treatment of EM/SJS/TEN has been controversial as their use may elicit systemic infection.\(^2,9\) The use of human immunoglobulin by intravenous injection for the treatment of canine SJS/TEN is thought to be beneficial for the treatment of the condition.\(^5\)

References

3. Hinn AC, Olivry T, Luther PB, Cannon AG, Yager JA. Erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis in the dog: Clinical presentation, drug exposure, and histopathologic correlations. Journal of Veterinary Allergy and Clinical Immunology 1998; 6:13-
EM, TEN, SJS: clinical signs and diagnosis: veterinary perspective

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The previously held view that these conditions were points on a continuum in human medicine is no longer held. Hinn et al. proposed a veterinary classification based quantitatively on areas of both skin and mucosae affected. This is, nevertheless, essentially a clinical severity scheme, albeit one that that differs from the human scheme.

1. Erythema Multiforme (EM) minor-target lesions, only one mucosal surface affected area affected, skin area less than 10%
2. EM major is clinically similar with more than one mucosal area affected, between 10 and 50% of skin area affected and less than 10% detachment
3. Stevens-Johnson syndrome (SJS) As above but greater than 50% of the body surface affected and up to 10-30% detachment.
4. Toxic epidermal necrolysis (TEN) was considered a separate syndrome with evidence of systemic illness and greater than 30% detachment.
5. TEN-SJS crossover has features of both syndromes but less that 30% detachment.

A lack of studies with sufficient case numbers has yet to validate the clinical relevance of this scheme. Multi-centre studies that can accumulate large numbers of cases are required to match pathogeneses, clinical signs, histopathology, treatments and prognosis with the individual classifications.

Clinical signs

Erythema multiforme
Lesions are usually polymorphous in dogs. While the classic target lesion of human erythema multiforme is occasionally seen, most targets are atypical in terms of the human definition. They may overlap, have ill-defined margins or appear polycyclic and may be raised or flat. Affected areas tend to be the trunk and especially the glabrous skin of the groin and axillae. This is different from human EM minor where lesions are generally acral and facial. Symmetry is evident in the lesions distribution of humans and dogs. Other commonly affected areas include the feet, ears, and oral cavity.

Evidence from Scott1 and observations from Ihrke suggests that target or targetoid lesions in dogs may evolve quite rapidly. Few clinicians may have had the opportunity to follow those changes in a significant number of dogs. Targetoid lesions may include urticarial wheals (implying dermal oedema) progressing to circular, annular and target lesions. Scott observes that the urticarial wheals of EM do not pit on pressure. Idiopathic “old dog” erythema multiforme may have relatively stable annular lesions.

However, EM does not manifest clinically just as targetoid lesions. There are descriptions of expanding and coalescing areas of erythema as well as epidermal detachment, erosions and bullae. Crusting is common. The presence of large areas of intensely erythematous to violaceous skin should also arouse suspicion for EM.

SJS-TEN and TEN-SJS overlap
Prodromal fever and malaise have been recorded in the dog. Erythema may start as a widespread macular eruption that progresses to confluent erythema. Atypical targets may also be present initially. Translucent epithelial sheets may slough. Pain is evident and a Nikolski-like sign is often present. Other lesions include ulcers, bullae, erosions and vesicles.

The face, footpads, oral cavity and mucocutaneous junctions are most commonly affected in dogs although the eye may also be affected. More widespread disease affecting the tracheobronchial and urogenital mucosae may be present in humans. Tracheobronchial lesions may be present in dogs.
Overall the lesions of EM and the SJS-TEN group could be regarded as a continuum with targetoid lesions more common in EM while bullae, erosions, ulcers and vesicles are more common in the SJS-TEN group.

In both EM and the SJS-TEN group the prognosis is related to the extent of the loss of mucosa and epidermis. In EM minor in humans the prognosis is good while the SJS in both groupings in both species is guarded to poor.

Differential diagnosis EM
Many dermatological conditions in dogs have targetoid lesions so there is a wide range of differentials including urticaria, dermatophytosis, drug reactions, superficial spreading pyoderma, epitheliotrophic lymphoma, the lupus diseases, eosinophilic granulomatous disease (Wells syndrome), superantigen dermatitis and neutrophilic dermatitis (Sweets syndrome) with hepato-cutaneous syndrome and the SJS-TEN group at the more severe end. The rapid progression of the latter to more severe signs should be a point of difference between the SJS-TEN group and EM.

Differential diagnosis of TEN, SJS, SJS-TEN overlap
These are the differential diagnosis of vesicles, bullae erosion and ulcers. They include a number of autoimmune diseases including pemphigus vulgaris, lupus, vasculitis, thermal injury, acquired epidermolysis bullosa and bullous pemphigoid and EM. Again the extent of the areas affected, the degree of mucosal involvement, the presence of a Nikolski-like sign and rapid progression of lesions would provide greater clinical suspicion for SJS-TEN group.

Diagnosis
As usual this is based on history, clinical signs, ruling out other causes of the specific lesions present and histopathology. Any history of neoplasia or illness should be considered relevant in EM. Vaccination, topical or systemic drug use may be more relevant for the SJS-TEN group. Histopathology will not reliably distinguish EM from a number of other diagnoses on its own but nevertheless offers vital support for this diagnosis. Biopsy sites should be intact erythematous skin or mucous membrane. Multiple sites should be sampled and ulcerated or crusted skin should be avoided. Submissions for histopathology should be marked “urgent” when SJS-TEN is suspected. Histopathology should be diagnostic for mature lesions of TEN in most canine cases.

Cats
The number of reported cases of EM in cats is insufficient to confirm a set of clinical signs but target lesions, exfoliation and erythema have all been recorded. Clinical signs of TEN appear similar to those in the dog.

Horses
EM is a rare disease of horses. Urticarial, targetoid and annular to serpiginous lesions may be distributed somewhat symmetrically over the trunk and do not usually crust. Mucus membrane lesions may also be present. It has been proposed that reticulated leukotrichia and hyperaesthetic leukotrichia are forms of EM5. Lesions frequently persist for weeks or months. Spontaneous recovery occurs in some horses and recurrence has been reported.
While presentations consistent with SJS have been recorded TEN appears to have yet to be described in the horse.

In all species the type and severity of clinical signs, their speed of progression and the areas affected will assist in identifying causes, prognosis and treatment.
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EM/SJS/TEN: overview – human perspective

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Introduction

Erythema multiforme (EM), Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are a group of mucocutaneous diseases characterized by varying degrees of skin and mucosal involvement. The latter two conditions associated with high morbidity and mortality.

The classification and terminology of these diseases has been constantly evolving, and new classification systems are being proposed every few years. Erythema exsudativum multiforme, as first described by von Hebra in 1866\(^1\), was later classified as either EM minor or major, the latter having mucous membrane involvement\(^2\). The terms EM Major and SJS were used interchangeably, which was quite confusing, even though the first cases reported by Stevens and Johnson in 1922 differed in many aspects\(^3\). The term TEN was introduced by Lyell in 1956\(^4\), and he agreed in a later paper that SJS and TEN are part of the same spectrum\(^5\).

It was previously thought that all these diseases were part of a single spectrum of disease; however, recent reports show that EM is now considered separate from SJS, SJS/TEN overlap, and TEN, in terms of aetiology and clinical features\(^6,7\). A consensus classification was introduced in 1993 by Bastuji-Garin et al., who proposed five categories: bullous EM, SJS, SJS/TEN overlap, TEN with spots, and TEN without spots\(^8\).

Erythema multiforme

Erythema multiforme (EM), as originally described by von Hebra in 1866, is a self-limited condition characterized by the abrupt onset of red papules which evolve into target lesions, with a tendency to recur. EM is classified into “EM minor” and “EM major” in an attempt to separate the classical, mild disease described by von Hebra (EM minor) which is most often associated with herpes simplex virus (HSV) infection in almost 50% of cases, and the more severe form with mucosal involvement usually attributed to *Mycoplasma pneumoniae* infections and drugs\(^9,10\).

EM may experience burning or itching at the site of the eruption, which usually appears symmetrically on the distal extremities, gradually progressing proximally. The involvement of the extensor surfaces of the extremities is common, as is involvement of the palms and soles. The individual lesions may start off as erythematous macules that later evolve into papules, plaques, and finally target lesions, which are only seen several days after the onset\(^10\). There are usually lesions of varying morphology coexisting, hence the term erythema “multiforme”. Target lesions have three distinct zones, a dusky area of central necrosis, a middle zone of pale oedema, and an outer zone of erythema\(^10\).

As EM is generally self-limiting, management rarely requires hospital admission. EM usually resolves spontaneously in three to five weeks, but has a tendency to recur\(^10\). This is usually the case when it is found in association with HSV infection. In these cases, there is a role for acyclovir prophylaxis.

Stevens-Johnson Syndrome

SJS is one of the rare, severe adverse cutaneous reactions to drugs, although a small proportion may be attributed to infection (i.e. HSV and *Mycoplasma pneumoniae*). As indicated in the criteria, SJS is characterized by erythematous or purpuric macules, widespread blisters predominantly on the chest, and involvement of at least 2 mucosal surfaces and less than 10% detachment\(^7,8\). Upon commencing treatment and withdrawal of the offending drug, re-epithelialization occurs, and there may be resulting post-inflammatory hyperpigmentation and scaling, with the average course of the disease lasting 2-3 weeks. Reported mortality is around 5%, and there are reports that prompt withdrawal of the offending drug reduces the risk of death by 30% per day, although this is generally in the case of drugs with short half-lives\(^11\). The management involves general supportive care measures, and referral to the ophthalmologist to prevent serious complications\(^12\).
**Toxic Epidermal Necrolysis**

TEN is a severe life-threatening disease characterised by mucosal involvement and greater than 30% epidermal detachment. Almost all cases are due to drugs, with allopurinol and carbamazepine being the most common causes in adults, and antibiotics and anticonvulsants in children. Similar to SJS, there is a prodrome of fever, malaise and mucositis followed by the onset of tender, purpuric target lesions and the extensive epidermal detachment that ensues within 24 hours, but most erupt over a period of two to fifteen days. The skin is usually quite painful to touch. Laboratory abnormalities include anemia and lymphopenia, with neutropaenia having a worse prognosis. There is also inflammation of internal mucosal surfaces such as the gastrointestinal and respiratory tracts due to the massive release of proinflammatory cytokines into the systemic circulation. This can lead to metabolic imbalance, multiorgan failure, pulmonary embolism, and gastrointestinal haemorrhage.

Due to the high mortality associated with TEN (30-50%), management of these cases usually require admission to burns or intensive care units, and prompt installation of supportive treatment and possibly IVIG or corticosteroids. The offending drug must be discontinued immediately, and supportive treatment must be initiated early to have the best outcome. Referral to the ophthalmologists paramount in order to prevent serious complications such as permanent visual loss due to corneal scarring or vascularisation.

**Overview of Management**

The cornerstone of treatment of EM, SJS, and TEN is meticulous skin care, fluid management, nutritional support, and surveillance as well as treatment of infections. When the precipitant is identifiable, treat the underlying cause, i.e. oral acyclovir in HSV-induced EM, or a macrolide in Mycoplasma-induced illness. In the case of drug-induced disease, the offending drug should be ceased.

General measures should be done, including increasing the room temperature to 30°C to 32°C to reduce caloric loss through the skin, giving anticoagulation with subcutaneous heparin or low molecular weight heparin to prevent deep vein thrombosis in immobile patients for the duration of their hospital stay. Pulmonary care is instituted via the use of aerosols, bronchial aspiration, and physical therapy. Various biologic dressings, as well as silver dressings due to their inherent antimicrobial properties have also been used.

There is really no established standard treatment for SJS and TEN. There have been numerous small studies and reviews suggesting that various treatments, in particular corticosteroids, are either beneficial or harmful. Data on therapy was retrospectively collected from patients in France and Germany enrolled in EuroSCAR, a case-control study looking at risk factors. It was found that neither IVIG nor corticosteroids had any significant effect on mortality in comparison with supportive care only, although a trend for beneficial effect of corticosteroids was noted. Currently, it is acceptable to use high dose systemic corticosteroids over a short period of time in SJS and TEN, and in addition, IVIG may be instituted early in TEN as small studies still show benefit.

**References**

IVIG: theory and practical use in human skin diseases

Dr. Lizbeth Intong, Dermatology Fellow
St. George Hospital, Sydney, Australia

IVIG: History

Intravenous Immunoglobulin (IVIG) was initially approved in the early 1980’s for the treatment of primary immunodeficiencies. It is now approved for other diseases such as myeloma, chronic lymphocytic leukaemia with severe secondary hypogammaglobulinaemia and recurrent bacterial infections, congenital AIDS, and immunomodulation for idiopathic thrombocytopaenic purpura, allogeneic bone marrow transplant, Guillain-Barre syndrome and Kawasaki disease. Its use in dermatology, however, has been purely off-label.

IVIG: Pharmacology

Plasma pools yielding IVIG range from 4,000 L to 15,000 L and contain plasma from 1,000 and 15,000 donors. Each aliquot of IVIG contains a polyclonal mixture of antibodies from different donors that imply that it contains multiple antigenic specificities. These pooled preparations are made up of more than 90% IgG and small amounts of IgA and IgM. There are several commercial preparations in Australia which are distributed as either lyophilised powders or liquid concentrates. The brands available here are Intragam P which comes in a solution for infusion (0.6g/10 mL, 3g/50 mL, 12g/200 mL), Octagam which also comes as a solution for infusion (2.5g/50 mL, 5g/100 mL, 10g/200 mL), and Sandoglobulin which comes as either a solution for infusion (6g/50 mL, 12g/100 mL) or as a lyophilised powder for reconstitution (6g reconstituted to 3% or 6% with NaCl 0.9% solution).

IVIG is a blood product and carries an inherent risk of transmitting infectious diseases so great care needs to be employed during donor selection and screening. An important thing to remember is that commercial IVIG products are not generic or identical. Each product has differences in volume load, osmolality, sodium content, sugar content, pH, and IgA content. This should be considered when selecting a product as small differences could affect clinical outcomes.

Indications in Human Skin diseases

IVIG has been used to treat a number of dermatological conditions (Table 1). There have been no large, multicenter, randomized, placebo-controlled trials reported as the diseases being treated are often rare and very severe. Most reports are small studies and case reports. The mechanism of action of IVIG is complex and poorly understood. There has been a decrease in serum levels of pathogenic auto antibodies noted after administration, with undetectable levels reached within 8-10 months. It has been shown that IVIG contains anti-Fas antibodies which can arrest apoptosis, hence its effectiveness in treating toxic epidermal necrolysis (TEN).

One of the most well reported uses of IVIG is in toxic epidermal necrolysis. It has been shown to be of benefit if given at a dose of 1-2g/kg/day for 4 consecutive days (3-5 days) both in adults and children. A brief review of the use of IVIG in the following dermatologic diseases will also be discussed during the lecture:

- Autoimmune Connective Tissue Diseases (dermatomyositis, lupus)
- Autoimmune Bullous Diseases (bullous pemphigoid, pemphigus vulgaris, mucous membrane pemphigoid)
- Drug-induced diseases (SJS/TEN)
- Vasculitis
- Urticaria
- Infections (Necrotizing fasciitis, streptococcal toxic shock syndrome)
- Atopic Dermatitis
**Table 1. Off-label applications of i.v. IG in dermatology**

<table>
<thead>
<tr>
<th>Application</th>
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<tbody>
<tr>
<td>Autoimmune dermatitis</td>
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<tr>
<td>Bullous pemphigoid</td>
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<td>Dermatomyositis</td>
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<tr>
<td>Epidermolysis bullosa</td>
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<tr>
<td>Pemphigus foliaceus</td>
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<tr>
<td>Pemphigus erythematosus</td>
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<td>Stevens-Johnson syndrome</td>
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<tr>
<td>Toxic epidermal necrolysis</td>
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<tr>
<td>Hyper-IgA syndrome</td>
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<tr>
<td>Hypereosinophilic syndrome</td>
</tr>
<tr>
<td>Mixed connective tissue disease</td>
</tr>
<tr>
<td>ANCA-positive vasculitis</td>
</tr>
<tr>
<td>Linear IgA dermatosis</td>
</tr>
<tr>
<td>Anti-neutrophil antibody syndromes</td>
</tr>
<tr>
<td>Pemphigus erythematosus</td>
</tr>
<tr>
<td>Pemphigus foliaceus</td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
</tr>
<tr>
<td>Molluscum contagiosum</td>
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<tr>
<td>Scleroderma derma</td>
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<tr>
<td>Scleroderma erythroderma</td>
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<tr>
<td>Scleroderma skin disease</td>
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<tr>
<td>Stevens-Johnson syndrome</td>
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<tr>
<td>Toxic epidermal necrolysis</td>
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<tr>
<td>Chronic urticaria</td>
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<tr>
<td>Varicella</td>
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<tr>
<td>Lyme disease</td>
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<td>Chang-Schmitz syndrome</td>
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<td>Wiskott-Aldrich syndrome</td>
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<td>Staphylococcal scald-skin syndrome</td>
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<td>Behcet's disease</td>
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<tr>
<td>Kaposi's sarcoma</td>
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<tr>
<td>Neutralizing St. Louis</td>
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</tbody>
</table>

Source: Fernandez and Kerdel (2007)

**References**

Management of EM/SJS/TEN: veterinary perspective

Dr. Peter Hill BVSc PhD DVD DipACVD DipECVD MRCVS MACVSc
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Although there is controversy about the pathogenesis of these conditions, and the relationships between them, the treatment strategy for managing these patients in veterinary medicine is essentially the same. The two main objectives are to:

- Stop the disease process
- Treat the damage that has already been done

Precisely what treatments are required will be dependent on the severity of the condition, and the clinical and histological findings.

Stopping the disease process

This may involve:

- Stopping previous medications
- Checking for, and treating, triggering infections
- Checking for, and treating, systemic diseases
- Possibly treating with drugs

Stopping previous medications is considered an essential step in the management of EM/SJS/TEN, especially considering that many such cases in animals are considered to be drug eruptions. When deciding whether or not to stop any medications the patient is receiving, the clinician needs to ask themselves what the impact will be of stopping those medications. For example, it may be feasible to safely stop a course of antibiotics without any major consequences. However, a dog that is receiving multiple drugs for the management of congestive heart failure is not likely to do well if those drugs are suddenly discontinued. Similarly, a dog that is receiving non-steroidal anti-inflammatory drugs to treat chronic arthritis is not likely to have a good quality of life if it has to live without them. Hence, the decision to stop medications must be based on a careful analysis of the severity of the new problem (the drug eruption) compared to the severity of the problem for which the patient was actually receiving the medication.

Checking for triggering infections is also important when managing these patients. At a simplistic level, the patient should be assessed for any obvious infections that could be detected on a physical examination e.g. ear infections, anal sac infections, oral infections, abscesses. However, such infections have rarely been implicated as a cause of EM/SJS/TEN, and even if they are present, it is difficult to ascertain if they are the primary cause of the skin disease. In many cases, it will be the antimicrobial agents that have been used to treat the infection that will have triggered the disease. In humans, although bacterial infections are known to be potential causes of EM, it is more typical for this condition to be triggered by viral (herpes) or mycoplasma infections. Detection of such infections in animals is more problematic without resorting to sophisticated tests that are not commercially available. Even with advanced methodology such as immunohistochemistry, it may not be possible to detect the viral fragments that can trigger the immune reaction in the skin.

Checking for systemic diseases is important for all these conditions. This is likely to involve blood tests and diagnostic imaging. If a suspected cause is found (e.g. a neoplasm), then prompt management may help the ongoing immune mediated damage to stop.

If an underlying cause can be found (drug, infection, tumour) and the condition is relatively mild, it may be acceptable to wait for spontaneous resolution to occur. However, for severe cases, specific intervention with drugs may be indicated. In humans, corticosteroids are not indicated for the treatment of EM, and their use in SJS and TEN is still controversial. Most authorities believe that they lead to increased morbidity and mortality in the more severe phenotypes. In animals, other factors need to be considered. The pathogenesis of these conditions in animals may not be the same as in people and the same contraindications may not apply. In cases of canine EM, there is often a T cell rich, interface dermatitis infiltrate into the superficial dermis. In the absence of a proven viral aetiology, the use of glucocorticoids would appear reasonably sensible. Also, the cost of these drugs may make them an attractive option for pet owners with a limited budget. In the more severe phenotypes, the use of glucocorticoids is harder to justify, especially if there is extensive ulceration and a cell poor infiltrate on histopathology. Clinicians must make a decision by
balancing the potential benefits (stopping the immune mediated reaction) against the potential risks (predisposing the animal to sepsis, reducing re-epithelialisation).

In the author’s opinion, cyclosporine would be the preferred option for the treatment of EM/SJS/TEN because it specifically inhibits the T cell mediated damage and is less likely to predispose to infection and inhibit epithelialisation. There is also some evidence that it is beneficial for the treatment of TEN in people. Pentoxyfylline can also be used for its immunomodulatory effects, either alone or in conjunction with cyclosporine or glucocorticoids.

In addition to targeting the lymphocyte infiltration, it may also be possible to inhibit the apoptosis that characterises EM and SJS. The administration of intravenous immunoglobulins can help to inhibit FAS ligand mediated apoptosis, thus reducing further disruption to epithelial integrity. Its use in animals suffering from EM or SJS has been reported. Cyclosporine may also have this beneficial effect.

**Treatment of the damage that has already been done**

This may involve:

- Correction and prevention of fluid losses
- Maintenance of adequate nutrition
- Treatment, and prevention, of secondary infection and sepsis
- Treatment of ulceration
- Provision of pain relief

Patients with extensive ulceration are at risk of severe metabolic complications. Intravenous fluid therapy and parenteral nutrition may be required. Prevention of secondary infection and sepsis is critical to ensure the patient’s survival. This is only necessary when there are large areas of epidermal loss. Antibacterial baths are indicated if the patient is well enough to withstand them. Systemic antibiotics are also likely to be needed in veterinary patients. Drugs such as fluoroquinolones or ticarcillin with activity against gram negative bacilli are indicated.

In human patients with TEN, the only intervention that has been proven to improve survival rates is transfer to a dedicated burns unit. These units have specific expertise in the management of metabolic complications and infection, as well as specialized dressings that are required to aid re-epithelialisation. In most cases, dressings combining Intrasite and Acticoat are used (both made by Smith & Nephew). Acticoat dressings contain a form of elemental sulphur that is gradually released from the dressing over time. This is highly effective in preventing colonization and infection by pathogenic bacteria. The application of Silver sulphadiazine cream may have a similar effect, but the sulphadiazine component may be contraindicated in patients with TEN if a drug eruption to sulphonamides is a possibility. In veterinary patients, the cost implications of adopting similar treatment strategies may be prohibitive for some owners, and a sub-optimal treatment regime may have to be accepted. In such cases, high mortality rates are likely to be the outcome.

The historical mortality rates in humans are approximately 5% for SJS and 40% for TEN. Sepsis and respiratory distress are the most common complications and ultimately the direct causes of death. Various factors influence the prognosis including the percentage loss of body surface area (BSA), age, persistent neutropenia (defined as neutropenia lasting >5 d), hypoalbuminemia (usually <2 g/dL), and persistent azotemia. However, with the advent of the newer dressing techniques, survival rates are improving, even with extensive areas of epidermal loss.

**Further reading**


Non-steroidal treatments for immune mediated disease: medical perspective

Dr. Lizbeth Intong, Dermatology Fellow, St. George Hospital, Sydney, Australia

Non-steroid Immunosuppressive Treatments

There are several non-steroid alternatives used to treat autoimmune diseases. Most of the time, however, these are just used as adjuvant treatments to systemic corticosteroids, which are still the mainstay of most of these conditions. We shall review the most commonly used non-steroid immunosuppressives used in dermatology.

Azathioprine

This is a synthetic purine analog is the prodrug of 6-mercaptopurine (6-MP) developed in the 1950’s initially as an antileukemic treatment. It is now widely used as an antiinflammatory and immunosuppressive agent across many specialties. It is one of the key medications in autoimmune bullous diseases, and is often added on to prednisone, and remains as a steroid-sparing agent once the prednisone has been tapered and discontinued. Table 1 shows a list of conditions in which azathioprine is known to be effective.

Azathioprine is available in Australia as Imuran, and comes as 50mg tablets. In bullous diseases, more commonly pemphigus, it is given at a dose of 2mg/kg/day up to a maximum of 3mg/kg/day. Therapeutic response can be observed after 6-8 weeks, but may take up to 12 weeks. Once the disease is stable, the dose may be decreased slowly. Prior to starting, it is important to do pre-screening of full blood count, renal function, liver function tests, as well as the thiopurine methyltransferase (TPMT) enzyme level. If this enzyme is low, it is not recommended to start the drug, as there is a high risk of acute bone marrow suppression. This test is available in Australia, and can be taken along with the other routine blood tests. A relative contraindication would be if the patient is on allopurinol as the drug interaction may lead to severe myelotoxicity. Alternatives like mycophenolate should be considered if azathioprine is not recommended.

Mycophenolate mofetil (MMF)

Mycophenolate was discovered in the 1970’s and initially used as a treatment for psoriasis. It is an ester derivative of mycophenolic acid, which is formed during fermentation of several *Penicillium* species, and has anti-inflammatory activity. It is mainly approved to prevent rejection of organ transplants. The main mechanism of action is selective inhibition of the purine biosynthesis enzyme inosine monophosphate dehydrogenase (IMPDH). It also inhibits lymphocyte and dendritic cell proliferation. Doses usually are 2g/day in combination with prednisone for pemphigus. There are two forms available in Australia, one is Cellcept, which comes in 250mg capsules, 500mg tablets or powder for oral suspension or solution for infusion. Another is Myfortic, which comes in tablets of either 180mg or 360mg (equivalent to 500mg).
It is generally well tolerated, and the main side effects are gastrointestinal (nausea, diarrhoea), and haematologic (leukopaenia). These are rapidly reversible with discontinuation of the drug. Fortnightly to monthly monitoring of full blood count, renal function, liver function tests is recommended during treatment. This is a safer alternative to azathioprine, the only setback would be the cost, as it is 5 times more expensive than azathioprine.

**Cyclosporin**

Cyclosporin (Neoral) is a calcineurin inhibitor that is very effective in treating immune-mediated inflammatory diseases, in particular, psoriasis, atopic dermatitis, and autoimmune bullous diseases. It was originally derived from a soil fungus *Tolypocladium inflatum gams* in the 1970's. Its main mechanism of action is inhibition of T-cell function. It also decreases release of histamine from mast cells. Cyclosporin is usually dosed at 3-4mg/kg/day, and gradually increased to 5mg/kg/day after 6-8 weeks if necessary. Inadequate response at 3 months is an indication for discontinuing treatment. It comes in 50 mg and 100 mg capsules. This is usually well-tolerated and side effects are rare. These include hypertension, deranged renal function, hypertrichosis, gingival hyperplasia, joint pains or headaches. Monitoring includes blood pressure monitoring as well as fortnightly full blood count, renal function, liver function tests. An algorithm is recommended in case of hypertension (Fig 1), and another on the management of renal toxicity (Fig 2).

![Fig 1. Management of Rising Blood Pressure](image-url)
Most side effects are reversible, and cyclosporin still remains one of the standard treatments in immune-mediated skin disease.

**Methotrexate**

Methotrexate (MTX) is a folic acid analogue that irreversibly and competitively inhibits dihydrofolate reductase. It is the second most common immunosuppressive agent prescribed by dermatologists after prednisone. It is used for a multitude of dermatology conditions including autoimmune bullous diseases, psoriasis, and atopic dermatitis (there are reports of successful use in children). It may be administered via IV, IM or orally. For most inflammatory disorders, the oral route is preferred as it has good oral bioavailability. Methotrexate is available as 2.5mg tablets. The usual dosage is 5-15mg/week. The tablets may be taken at the same time, but some doctors prefer to give it in 3 doses at 12 hours apart. It is also now standard practice to give folic acid 5mg the next day to reduce the deleterious side effects of the drug. Prior to starting MTX, pre-screening of full blood count, renal function, liver function tests, HIV, hepatitis screening should be performed as this drug may cause hepatotoxicity. Pregnancy is an absolute contraindication as this drug is teratogenic. Table 3 lists the contraindications to MTX.
During treatment, fortnightly blood tests (FBC, EUC, LFT, beta HCG if female of childbearing age) should be ordered to monitor for changes. Table 5 lists the guidelines for hepatotoxicity monitoring.

**Table 3. Monitoring for hepatotoxicity in paediatric patients: two alternative approaches.**

1. **AMO Guidelines (86).**
   a. Low-risk patients:
      i. Liver biopsy every 1 to 1.5 mg/kg of therapy in low-risk patients.
      ii. After a cumulative dose of 4 g/kg, biopsy after each 1 g/kg of therapy.
    b. High-risk patients:
      i. Consider delayed baseline liver biopsy after 2–3 months of therapy, to establish medication efficacy and tolerability in high-risk patients.
      ii. Repeat liver biopsy every 2.5–3 g/kg of therapy.
      iii. After abnormal biopsy results (Table 5):
         1. Biopsy within 1 month, if abnormal
         2. If histologic grades III/IV, consider alternative therapy.
   c. For histologic grades IIb and IV, discontinue therapy.

2. **Mucolymph guidelines (355).**
   a. Baseline PHCNPs levels (if possible):
      i. Repeat PHCNPs every 2–3 months while on therapy.
   b. Indications for considering a liver biopsy:
      i. Persistent PHCNPs > 0.5 μg/L.
      ii. An increase in abnormal PHCNPs levels > 1.0 μg/L over a 1.5-month period.
      iii. Elevated PHCNPs level above 5.0 μg/L in two consecutive samples.
   c. Indications for considering withdrawal of therapy:
      i. Elevated PHCNPs level > 10.0 μg/L in three consecutive samples in 1.5 months.
      ii. The decision whether to perform a liver biopsy, withdraw medication or continue treatment, despite elevated PHCNPs levels must take into account factors such as disease severity, patient age, and the ease with which alternative therapies may be used in place of methotrexate.

MTX has been used successfully for over 40 years in dermatology, and with careful monitoring, is an important cost-effective tool in treating many immune-mediated skin diseases.

**Hydroxychloroquine (Plaquenil)**

Hydroxychloroquine is available for oral administration as 200mg capsules and is the form of hydroxychloroquine sulfate (Plaquenil). The main mechanism of action of antimalarials is interference with lysosomal acidification within dendritic cells, macrophages, and lymphocytes which ultimately results in their anti-inflammatory effect. They have been shown to enhance the protective early limb of UV response through up-regulation of c-Jun.

**Table 3. Contraindications to methotrexate therapy**

<table>
<thead>
<tr>
<th>Contraindications</th>
<th>No dose adjustment</th>
<th>Reduced dose by 50%, more frequent monitoring</th>
<th>Tapering of methotrexate</th>
<th>Drug contraindicated</th>
<th>Last 4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal dysfunction</td>
<td>Mild renal insufficiency (GFR &gt; 90 ml/min)</td>
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<td></td>
<td>Moderate renal insufficiency (GFR 60–89 ml/min)</td>
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<td></td>
<td>Severe renal insufficiency (GFR &lt; 60 ml/min) or ESRD or dialysis</td>
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<td></td>
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<tr>
<td></td>
<td>Significant liver dysfunction</td>
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<td></td>
<td>Hepatopathy</td>
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<td></td>
<td>Cytomegaviol</td>
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<td>Alcoholic or heavy alcohol consumption</td>
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<td>Obesitiy</td>
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<td></td>
<td>Abruption or thrombocytopenia or leucopenia (Severe)</td>
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<td></td>
<td>Uncontrolled hypertension</td>
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<td></td>
<td>Absolute contraindications (Uncontrolled or unmonitored)</td>
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<tr>
<td></td>
<td>Pregnancy category of uncontrolled</td>
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*Dermatology Chapter of the ACVSc Science Week Proceedings, Gold Coast, 2-3 July 2010*
The main indication is for photosensitive disorders such as lupus erythematosus (400mg/day for at least 2 months), porphyria cutanea tarda (100mg 2 times weekly), dermatomyositis, solar urticaria, and polymorphous light eruption.

**Dapsone**

Dapsone or diaminodiphenylsulfone is one of the oldest drugs in dermatology. It was first synthesized in 1908, and was initially used for its antibacterial properties (e.g. leprosy). Over the years, it has been used to treat a whole spectrum of dermatologic diseases. It was found to be of value in autoimmune bullous diseases, in particular in those with predominant neutrophilic infiltrates like linear IgA disease and dermatitis herpetiformis. Currently, it is TGA and FDA-approved for the treatment of leprosy and dermatitis herpetiformis.

The exact anti-inflammatory mechanism is not known, but it has been shown to suppress non-specific inflammation in a manner similar to corticosteroids. It suppresses myeloperoxidase generation of free radicals from neutrophils, which could explain why it is effective in neutrophilic skin diseases.

It comes in 25mg and 100mg tablets, and it is usually recommended to start at a low dose (i.e. 50mg/day) increased slowly by 50mg/week until disease control is attained.

Prior to starting, pre-screening should include full blood count with reticulocyte count, EUC, LFT, including G6PD level as the most common side effects are hematologic (hemolysis, methemoglobinaemia, leukopenia, agranulocytosis). These are usually dosage-related, and is more pronounced in patients with deficiency of this enzyme. Those with low G6PD levels should not be commenced on dapsone. Neurologic function must also be monitored as dapsone may cause peripheral neuropathy due to axonal damage if given at high doses. Another important complication is dapsone hypersensitivity syndrome, which presents with fever, malaise, lymphadenopathy, a morbilliform rash, hepatomegaly and jaundice. This can occur 5-6 weeks after starting dapsone, and can be fatal if untreated.

**Tetracycline/Nicotinamide**

The combination of tetracycline and nicotinamide for the treatment of bullous pemphigoid (BP) was first reported in 1986. Although the mode of action is unclear and is presumably due to the anti-inflammatory effect of tetracycline, this has been confirmed by several studies. One study in 1995 reported a regimen of 2g tetracycline combined with 2g nicotinamide daily was effecting in clearing lesions and inducing remission within 6-8 weeks. The mean duration of treatment was seven months. Another study reported the results of a randomized, open label trial comparing a combination of nicotinamide 1500mg nicotinamide and 2g tetracycline daily with prednisone therapy in 20 patients with BP. All five patients in the nicotinamide and tetracycline group receiving long-term follow-up remained disease free during medication tapering, while three patients in the prednisone group had repeated disease flare-ups with steroid tapering. There were fewer side-effects in the first group, as compared to the steroid group. The combination of nicotinamide and tetracycline appears to be a useful alternative to systemic steroids in the treatment of bullous pemphigoid. This would be useful in patients with localised disease and who have complicated medical conditions where long-term corticosteroid use is not ideal. Currently, newer tetracyclines such as doxycycline 100 mg bd or minocycline 50-100 mg bd can be substituted for tetracycline.

**Other immunosuppressive agents used in immune-mediated disease**

The lecture will also touch briefly on other new and less commonly used immunosuppressives including cyclophosphamide, rituximab (CD20+ B cell antagonist used successfully in pemphigus), and biologics (etanercept, infliximab, adalimumab).

**References**

Non-steroidal treatment options for immune mediated disease –
 veterinary perspective

Dr. Mike Shipstone, Dermatology for Animals
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Introduction
Depending on their mode of action immunosuppressive drugs can be classified into four different groups:
• Antinflammatory drugs of the corticosteroid group
• Inhibitors of the calcineurin pathway
• Cytotoxic or antiproliferative drugs
• Specific antibodies

These notes will be limited to those compounds that have been reported useful in the treatment of cutaneous
immune mediated disease. Those covered include: Vitamin B3/tetracycline combination, Cyclosporine,
Mycophenolate mofetil, Intravenous immunoglobulins, Chrysotherapy and Dapsone. Azathioprine and
Chlorambucil have been used commonly but will be covered in depth by another speaker and so will not be
examined here.

Vitamin B3 (nicotinamide) / Tetracycline Combination
Mode of Action
Tetracycline can inhibit in vitro antibody production, inhibits compliment activation, prostaglandin synthesis
and action of lipases and collagenases, decreases leucocyte chemotaxis. Niacinamide blocks IgE induced
histamine release, prevents mast cell degranulation, decreases protease release and inhibits phosphodiesterase.
Whilst in vitro antibody production is affected by tetracycline a study of the long-term use of the
combination in DLE failed to show any effect on the in vivo antibody production.

Side Effects
Anorexia, lethargy, vomiting, diarrhoea, increased seizure activity have been reported in the dog. In humans
reported side effects include vertigo, vestibular disturbance, tinnitus, ataxia and neuromuscular blockade.

Indications
The combination of tetracycline/Niacinamide or Doxycycline/Niacinamide have been reported as effective in
the treatment of DLE, Lupoid onychitis, cutaneous reactive histiocytosis and pemphigus. Whilst it may be
used as a sole therapy in some conditions in the authors experience it seems to be less effective and is an
adjunctive therapy at best in pemphigus.

Dose: 500 mg of each drug Q 8 h if > 10 kg, 250 mg of each drug if <10 kg. Due to the difficulty of owner
compliance with TID dosing the author uses the following combinations: Doxycycline 5 mg/kg Q 12h with
Niacinamide 500 mg Q 12h >10 kg, 250mg Q 12h <10 kg. In larger dogs the Niacinamide dose may be
further increased

Cyclosporine
Mode of Action
Cyclosporine gains entry in the cells by a nonspecific association with membrane phospholipids, it
then binds with specific recognition in T cell to cyclophilin. The cyclosporine/cyclophilin complex
binds to a target protein - Calcineurin A.
Normally calcineurin A dephosphorylates a cytosolic protein - nuclear factor of activated T cells (NF-AT). The dephosphorylated NF-AT is transported to the nucleus, binds to promoter region of
various cytokine genes leading to cytokine production. The cyclosporine/cyclophilin binding thus
inhibits dephosphorylation of NF-AT by calcineurin & transport to the nucleus. THUS cytokines
are NOT produced. Specificity of cyclosporine for T-cells may be due to lower levels of
calcineurin in T cells than other cells

The net results of this action is inhibited transcription of:
• IL-2 leading to impaired proliferation of activated T-helper & T-cytotoxic lymphocytes
• IFN-α which provides amplification signals for macrophage & monocyte activation
This early inhibition of T cells also causes a number of other actions
• Inhibited production other cytokines inc IL-3,4,5, TNF-α, IFN-γ,
• Inhibits mononuclear cell function, antigen presentation, mast cell & eosinophil production, histamine release from mast cells, neutrophil adherence, NK cell activity, growth and differentiation of B-cells.

**Side Effects**
Adverse reactions consist mainly of transient emesis and diarrhoea, which seems to during the first days of treatment. However in some instances the emesis may be so severe as to prevent continued drug administration. Other adverse reactions, such as gingival hyperplasia, verruciform lesions and hypertrichosis, appear to be dose-dependent, and occur rarely at therapeutic doses. An increased susceptibility to infections has not been reported in dogs receiving this drug.

**Indications**
It has been clearly shown that Cyclosporine has good and repeatable efficacy in treating some immune mediated diseases including canine atopic dermatitis and ulcerative anal furunculosis. The dose most commonly used for atopy is 5 mg/kg Q 24 h, for one month and then dose titration to establish lowest effective dose. The dose used for anal furunculosis is generally higher, 4 mg/kg Q 12h has been reported to give resolution or reduction in 25 of 26 dogs treated.

There have been a number of case reports of the use of cyclosporine for treatment of pemphigus. An occasional individual has shown improvement (Rosenkrantz 2004), with other studies showing no effect (Olivry et al 2003). It should be noted that Rozenkrantz recommends a higher dose (10 mg/kg Q 24h) given in conjunction with ketoconazole (5 mg/kg Q 24h ) which will have the effect of further raising serum concentrations because of the competitive inhibition of the hepatic metabolism of the two drugs.

**Mycophenolate mofetil (MM)**

**Mode of Action**
MM causes reversible inhibition of a key enzyme (inosine monophosphate dehydrogenase) in the de novo (from scratch) synthetic pathway of guanine nucleotides and the incorporation of purine into DNA. B & T lymphocytes are dependent on this synthetic pathway for the production of guanosine because they are unable to use the salvage guanosine pathway.

The effect of this is the inhibition of clonal expansion of the lymphocytes and reduction in primary (not secondary) antibody production. Other immunosuppressive effects include induction of apoptosis in activated T cells and suppression of glycosylation leading to inhibition of adhesion molecule expression and lymphocyte recruitment. As other tissues are able to bypass this inhibition (through use of the salvage pathway), effects on other tissues is low and efficacy: toxicity ratio is good.

**Side Effects**
Bone marrow suppression, nausea, vomiting, diarrhoea, increased incidence of infections (in humans)

**Indications**
It has been sporadically reported for a number of different diseases including pemphigus, myasthenia, aplastic anaemia, necrotizing meningioencephalitis and immune mediated glomerulonephritis.

Doses range from 22 to 39 mg/kg/24 h divided every 8 h

The author has used it on a number of occasions for the treatment of pemphigus with mixed (generally poor) response.

**Chrysotherapy**

**Mode of Action**
Anti-inflammatory, antirheumatic, immunomodulating & antimicrobial (in vitro) effects

1. Reduces release of inflammatory mediators (lysosomal enzymes, prostaglandin's, histamine) and inhibits a number of enzymes (esp. lysosomal enzymes) It enters the lysosomes of phagocytic cells and induces an alteration of lysosomal structures.
2. Inhibitory effect on the first component of complement in vivo
3. Inhibit the chemotactic and phagocytic responses of macrophages and polymorphs in vitro and block PMN degranulation in vitro, but not in vivo
4. Interferes with immunoglobulin-synthesizing cells.

The decline in pemphigus antibodies during parenteral chrysotherapy is probably rather a secondary phenomenon

5. Oral gold has been shown to be a potent inhibitor of antibody-dependent, cell-mediated toxicity and complement lysis in vitro and thus would suppress both antibody production and response. It also can suppress the response to skin test and may stimulate suppressor T-cells
6. Oral gold also has an inhibitory effect on DNA- RNA- and protein synthesis in vitro
7. Small metal compounds such as gold could also potentially block the autoimmune response by disrupting the MHC-peptide interaction (can strip peptides from a human MHC-II protein by an
allosteric mechanism) and in addition, block the ability of antigen-presenting cells to activate T-cells.

NB whilst all of these actions have been identified, its exact mechanism of action in vivo still remains unclear.

Gold is available in 2 forms: an oral compound Auranofin and an injectable form sodium aurothiomalate (Myocrisin®). An older injectable compound, aurothioglucose, is no longer available. It was this compound that most veterinary references report.

**Side Effects**
Skin rashes (need to test dose to see if sensitive), proteinuria, bone marrow suppression, oral ulceration, glomerulonephropathy and once case of fatal TEN when dogs were switched from Azathioprine to gold salts without a wash out period

**Indications**
Gold salts have been reported in the treatment of pemphigus foliaceus, pemphigus vulgaris and rheumatoid arthritis in dogs and pemphigus in cats.

The injectable forms have a long lag phase (10 – 16 weeks)

Dose: 1mg/kg Q 7 until remission and then Q 4 weeks to maintain remission.

Oral: 0.05 – 0.2 mg/kg Q 12h

Monitoring: CBC/platelet counts Q 7 d for first 4 weeks, then Q 4 weeks to 4 months then Q 3 months. Periodic MBA, UA (Q6 weeks for first 4 months then Q 6 m.

**Dapsone**

**Mode of Action**
Inhibits neutrophil chemotaxis selectively induced by a bacterial product but NOT leukocyte derived chemotactic factor or C5a. It decreases neutrophil lysosomal activity, inhibits cyclo-oxygenase and thus conversion of arachidonic acid to PG’s, inhibits neutrophil adherence to basement membrane zone antibodies (IgA & IgG) in a dose dependent manner. It does not appear to affect neutrophil phagocytosis, antibody deposition in tissue, serum Ab levels or complement activation

THUS: useful in conditions of excess neutrophil accumulation: linear IgA, leukocytoclastic vasculitis, bullous LE, BP, subcorneal pustular dermatosis

**Side Effects**

**Humans**, anemia, methemoglobinemia, agranulocytosis, hepatitis, cholestatic jaundice, headaches and peripheral neuropathy have been reported. In all instances, these side effects were reversible. Dogs: potentially, rapid onset of profound leucopenia (normal within 10 d of cessation). Mild anaemia, leucopenia, moderate elevation of SAT (may be expected to a mild degree during initiation, do not stop treatment if patient is clinically normal).

Blood dyscrasias, thrombocytopenia, skin reactions (drug eruptions) and hepatic toxicity can be serious.

Patients should be monitored by CBC, UA, BUN and ALT every two weeks during induction.

**Cats** especially susceptible with increased incidence of hemolytic anemia and neurotoxicity’s and so should be AVOIDED.

**Dose:**
1mg/kg Q 8 h

**Intravenous immunoglobulin (IVIG) therapy**

**Mode of Action**
IVIG is a highly purified IgG preparation made from the pooled plasma of normal patients and contains ≥ 95% IgG.

A diverse range of mechanisms has been proposed and it is likely that the immunomodulatory effects are affected by several different paths, which seem to act in concert. Anti-inflammatory mechanisms of actions include the following:

- Neutralisation of autoantibodies
- Inhibition of compliment binding and activation
- Effects mediated by Fc receptor binding
- Enhancing clearance of pathogenic autoantibodies via saturation of the neonatal (FcRn) salvage pathway
- Suppression of pathogenic cytokines
• Down regulation of T or B cell function.

Side Effects

Humans: headache, myalgia, flushing, nausea, tachycardia. Severe anaphylactic reactions have been reported (mechanism unclear but seems to be dependent on the IgA concentration in the IVIG, anti-IgA activity in patient, infusion speed and Tx interval) as have rare reports of acute renal failure (osmotic nephrosis) associated with sugar additives used to stabilize the preparation.

Canine: as there is no canine IVIG available human (or equine) products must be used. These are foreign proteins and as such cannot be used repeatedly due to the risk of acute anaphylaxis.

Indications.

In humans IVIG may be used as replacement therapy at a dose of 300-500 µg/kg to achieve serum levels of 500 mg/dl which must be repeated Q 3-4 weeks to maintain protective Ab levels. It may also be used as an anti-inflammatory agent at a dose of 1-3 g/kg body weight. It has been used in a wide range of diseases in Australia (Table 1). Its use has increased from 20 gm/1000 population in 1994/95 to 100 gm/1000 in 2006/07 and has been estimated that 50-60% of its use worldwide is “off label”.

Dermatological conditions that have been treated with IVIG in Australia include: Dermatomyositis, Kawasaki disease, Bullous pemphigoid, Cicatricial (mucous membrane) pemphigoid, Pemphigus vulgaris, Pemphigus foliaceus, IgA pemphigus, Toxic epidermal necrosis (TEN)/Steven-Johnson Syndrome, Epidermolysis Bullosa aquisita.

Canine Use.

Few reports of IVIG use in animals exist but diseases in which it has been used include immune mediated thrombocytopenia, immune mediated haemolytic anaemia, myelofibrosis, pemphigus foliaceus and adverse drug reactions.

IVIG is given at a dose of 1 g/kg over a 6 – 12 hour period and may be repeated in 24 hours.
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Open Forum: Non-steroidal management of immune-mediated skin disease

Chair: Beth McDonald
Mike Shipstone, David Robson, Allan Bell, Peter Hill

Notes:
## Case Reports and Research Projects

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Skin barrier function
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The importance of skin barrier function for various skin disorders has been increasingly recognised over the last 10 years in human and veterinary medicine. Barrier dysfunction is a typical feature of human atopic dermatitis (AD) and allergic diseases, allowing for the penetration of allergens, microorganisms and other irritants into the skin and eliciting inflammation, bacterial colonization and allergen sensitization. The epidermis functions as the primary defence layer to the external environment, and includes epidermal cells and a cornified layer, but most of the barrier function is regulated by the stratum corneum (SC) and the tight junction which resides at the level of the stratum granulosum. When the SC is compromised, usually due to decreased levels of SC lipids, mechanical trauma resulting from extensive scratching that is precipitated by an intensive itching sensation is observed.

SC lipids in skin barrier function
In humans, several studies have suggested that reduced levels of ceramides, the major constituents of intercellular lipids in the stratum corneum are involved in defective barrier function of AD. 

Lower levels of ceramides in the SC are thought to accelerate transepidermal water loss (TEWL) and decrease water capacitance resulting in atopic dry skin.

Additionally, impairment of the barrier function of the SC may facilitate percutaneous entry of aeroallergens that might provoke an immune reaction. Thus, the loss of barrier function in the SC might be one of the key roles in the development and aggravation of AD in humans.

In dogs, electron microscopic analysis revealed that the continuity and the thickness of the intercellular lipid lamellae are significantly reduced in non-lesional atopic skin when compared with normal canines. Alternatively, Chesney reported that the skin hydration and water dynamics of the skin of atopic dogs did not differ from those of normal dogs. Currently, the association of altered lipid barrier function in the pathophysiology of canine AD is not fully understood.

Recently, a new method of measuring TEWL on canine skin with a closed chamber device showed less variable measurements than the conventional open-chamber method. Furthermore, it was reported that TEWL was increased in canine skin by disruption of the barrier function via tape stripping. Thus, TEWL measured by a closed chamber device is thought to reflect the skin barrier function in dogs as well as in humans. TEWL, in both lesional and non-lesional skin of dogs with atopic dermatitis, exhibited significantly high values compared with non-atopic dog skin. This result may indicate that canine atopic dermatitis is possibly to relate to decreased skin barrier function.

Ceramides make up approximately 30% of skin lipids and rest of skin lipids are cholesters(60%) and free fatty acids (10%) in normal dog skin. Ceramides are composites of sphingosine and fatty acids, and are capable of holding a large amount of water through the OH- terminal. Therefore, a defect in ceramides of the SC may elicit skin dryness thereby leading to sensitive skin.

In a previous study, a topical lipid preparation containing ceramides, free fatty acids and cholesterol was applied to five dogs with atopic dermatitis and after repeated application of the lipid preparation, the lamellar layer of the SC was observed to have increased. The decreased proportion of ceramide in lesional and non-lesional skin of dogs with atopy has been reported. This study demonstrated a decreased ratio of ceramide to free-fatty acids and cholesterol. There were negative correlations between relative ceramide amounts and TEWL suggesting decreased ceramides may elicit dryness of skin and skin barrier dysfunction. Reiter et al. demonstrated that decreased levels of specific ceramide subclasses 1 and 9 might be involved in impaired barrier function in canine atopic dermatitis.

Filaggrin mutations in human and canine atopic dermatitis
Atopic dermatitis in humans and dogs has been regarded as a genetic disorder with a familial occurrence or breed predilection. It is believed that skin barrier dysfunction is an essential feature of atopic dermatitis and allergic diseases in general. In human atopic dermatitis, it is reported that 12.5–48% of patients have mutations in filaggrin. Thus, a loss-of-function filaggrin mutation is thought to be a risk factor for atopic dermatitis in humans.

Marsella et al. compared immunohistochemical staining of filaggrin between normal and house dust mite sensitized beagles and found no obvious correlation between immunostaining and clinical scores. Recently,
a linkage analysis was investigated in non-atopic and atopic West Highland White Terriers (WHWT) and in this study, a large causative role of the flg orthologue in atopy was excluded.\textsuperscript{12} There are no studies demonstrating an obvious relationship between flg mutations and risk of atopic dermatitis in dogs, but future studies should hopefully elucidate the degree to which skin barrier function contributes to the pathogenesis of canine atopic dermatitis.

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