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

Dr. Mike Shipstone, Dermatology for Animals
263 Appleby Rd
Stafford Heights, 4053

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 phophodiesterase. 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 synthesis 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 rash (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 4-21 d for 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”.

Table 1 Clinical conditions in which treatment with IVIG has approval (from Smith et al 2010)

<table>
<thead>
<tr>
<th>Conditions for which IVIG has an established therapeutic role</th>
<th>Conditions for which IVIG has an emerging therapeutic role</th>
<th>Conditions for which IVIG use is in exceptional circumstances only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replacement therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acquired hypogammaglobulinaemia</td>
<td>Secondary hypogammaglobulinaemia</td>
<td>Epidermolysis bullosa acquisita</td>
</tr>
<tr>
<td>secondary to haematological malignancies</td>
<td></td>
<td>Acute leukaemia in children</td>
</tr>
<tr>
<td>Primary immunodeficiency diseases</td>
<td>Specific antibody deficiency</td>
<td>Autoimmune congheart block</td>
</tr>
<tr>
<td>Immunomodulatory therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory myopathies</td>
<td>Bullous pemphigoid</td>
<td></td>
</tr>
<tr>
<td>Kawasaki disease</td>
<td>Cicatrical pemphigoid</td>
<td></td>
</tr>
<tr>
<td>Chronic inflammatory demyelinating polyneuropathy</td>
<td>Pemphigus foliaceus</td>
<td></td>
</tr>
<tr>
<td>ITP in adults (including thrombocytopenia arising as a result of HIV infection)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guillain–Barré syndrome</td>
<td>Pemphigus vulgaris</td>
<td>Autoimmune diabetic neuropathy</td>
</tr>
<tr>
<td>Lambert–Eaton myasthenic syndrome</td>
<td>Toxic epidermal necrosis/Stevens-Johnson syndrome</td>
<td>Autoimmune neuropenia</td>
</tr>
<tr>
<td>Multifocal motor neuropathy</td>
<td>Acute disseminated encephalomyelitis</td>
<td>Catastrophic antiphospholipid syn</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>ANCA-positive necrotising vasculitis</td>
<td>Coagulation factor inhibitors</td>
</tr>
<tr>
<td>Neonatal haemochromatosis</td>
<td>Autoimmune haemolytic anaemia</td>
<td>Devic dz (neuromyelitis optica)</td>
</tr>
<tr>
<td>Stiff person syndrome</td>
<td>Evans syndrome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Feto-maternal/neoatal alloimmune thrombocytopenia</td>
<td>Epilepsy</td>
</tr>
<tr>
<td></td>
<td>Haemophagocytic syndrome</td>
<td>Graves ophthalmopathy</td>
</tr>
<tr>
<td></td>
<td>High-risk alloigenic haemopoietic stem cell transplantation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ITP in children</td>
<td>Haemolytic disease of the newborn</td>
</tr>
<tr>
<td></td>
<td>Immunoglobulin M paraproteinaemic neuropathy</td>
<td>Haemolytic transfusion reaction</td>
</tr>
<tr>
<td></td>
<td>Kidney transplantation</td>
<td>HIV in children</td>
</tr>
<tr>
<td></td>
<td>Multiple sclerosis</td>
<td>Myocarditis in children</td>
</tr>
<tr>
<td></td>
<td>Opsoclonus myoclonus ataxia</td>
<td>PANDAS</td>
</tr>
<tr>
<td></td>
<td>Post-transfusion purpura</td>
<td>Paraneoplastic syndromes</td>
</tr>
<tr>
<td></td>
<td>Toxic shock syndrome</td>
<td>Potassium channel antibody-associated encephalopathy</td>
</tr>
</tbody>
</table>

Dermatological conditions that have been treated with IVIG in Australia include: Dermatomyositis, Kawasaki disease, Bullous pemphigoid, Cicatrical (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.
References

Liberman AC, Druker J, Refojo D, Arzt E. Molecular mechanisms of some immunosuppressive drugs. 2008 Medicina. 68(6):455-64


Yuki et al. Recovery of dog from aplastic anaemia after treatment with MM. 2007. AVJ 85(12): 495-497


