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 neutropenia 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 arachidonic 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 challenges 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. 3

**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. 3

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). 3 It has also been more recently used in combination with prednisolone for the treatment of meningococcophalomyelitis of unknown aetiology, at a dose rate of 50mg/m² every 12 hours for 48 hours, repeated every three weeks. 3 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 ribonucleotide 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. 3 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. 1,3

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. 1,3

**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. 3,4 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. 3

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). 3 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. 3,4

**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. 3,4

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-calmodulin complexes, which impede calcium dependant signal transduction. The calcineurin-calmodulin 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.\textsuperscript{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.\textsuperscript{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 thrombocytopenia, but repeated administration could increase the risk of hypersensitivity reactions.\textsuperscript{3} The high cost of the therapy can also be a limiting factor.

References