Several potent immunosuppressive drugs developed over the past few decades in human medicine have recently made the leap to our small animal patients, and our use of them is growing. This lecture will discuss cyclosporine, leflunomide and mycophenolate.

Cyclosporine

Cyclosporine is a potent immunosuppressive drug indicated for the treatment of inflammatory and immune-mediated diseases, and for organ transplantation. Cyclosporins are cyclic polypeptide macrolides originally derived from the soil fungus *Beauveria nivea* (*Tolypocladium inflatum*), but are also produced by other fungal organisms. Cyclosporine A is the molecule developed for commercial use as an immunosuppressive agent. Discovered in the 1970s, the use of cyclosporine as an immunosuppressive agent was first described in humans to prevent rejection of renal allografts. Within a decade, cyclosporine had become the cornerstone of immunosuppression for organ transplantation. In veterinary medicine, oral cyclosporine capsules received FDA approval in 2003 for the treatment of canine atopy, and were more recently also approved for allergic skin disease in cats. Cyclosporine has been used in an extra-label fashion for many years for renal transplantation in dogs and cats, and for the treatment of a variety of inflammatory and immune-mediated conditions.

Cyclosporine’s primary immunosuppressive mechanism of action is inhibition of T lymphocyte function. Cyclosporine acts to inhibit calcineurin, an intracellular protein phosphatase that activates gene transcription factors through dephosphorylation. In the untreated patient, activation of T cells results in activation of calcineurin, which dephosphorylates inactive nuclear factor (NFAT). NFAT translocates into the nucleus, where it upregulates transcription of genes coding for several important cytokines, including IL-2, IL-4, TNF-α, and INF-γ. Production of IL-2 in particular plays a key role in the activation and proliferation of T cells. Calcineurin inhibitors, including cyclosporine, act by binding to intracellular cyclophilins, which are proteins that facilitate protein folding. Binding of cyclosporine to cyclophilin A creates a complex with high affinity for calcineurin. Through inhibition of calcineurin, cyclosporine specifically inhibits T cell function and thus, cell-mediated immunity, but has little immediate impact on humoral immunity. Decreased IL-2 expression in CD4+ Th1 cells associated with cyclosporine therapy leads to inhibition of proliferation and activation of both T-helper and T-cytotoxic lymphocytes, and blunting of the immune response. Cyclosporine has also been shown to have many other local anti-inflammatory and immunosuppressive effects, especially in the skin.

Cyclosporine is a large lipophilic molecule which must be solubilized prior to intestinal absorption. Commercial cyclosporine is available as two very different types of oral formulations. Cyclosporine was initially approved in humans as a vegetable-oil based preparation (Sandimmune®), but variability in oral bioavailability caused marked variability in blood drug concentrations. A more recent formulation, an ultramicronized preparation approved
in 1996 (Neoral®), forms a microemulsion upon contact with aqueous fluids, resulting in more consistent and predictable absorption. Oral bioavailability of the microemulsion is improved by up to 50% compared to the oil-based preparation. Because of the marked variability in bioavailability of the non-ultramicronized preparation, it is not recommended for oral use in small animals.

Cyclosporine has a high binding affinity for red blood cells and plasma lipoproteins. Because up to 50% of the drug in blood is located in red cells, whole blood is recommended for therapeutic drug monitoring (TDM). Once in the circulation, cyclosporine distributes widely, accumulating in the skin, liver, kidneys, and fat of dogs, resulting in a large volume of distribution. Tissue levels exceed levels in serum by a factor of 3 to 14. Peak blood concentrations generally occurring approximately 2 hours after oral administration of cyclosporine. Blood concentrations then rapidly decrease over the remainder of the dosing interval, reflecting a relatively rapid half-life as the drug is cleared from plasma.

Extensive metabolism of cyclosporine by the hepatic cytochrome P-450 system yields many different metabolites, some of which may retain therapeutic efficacy. In dogs, several drugs that inhibit P-450 enzymes have been given concurrently with cyclosporine in order to decrease the dose needed to maintain adequate blood drug concentrations. Ketoconazole, in particular, has been used to decrease in oral cyclosporine dosages in dogs by as much as 75 percent, although individual responses are variable.

The complexities of cyclosporine disposition in normal animals, coupled with confounding factors associated with disease and differences in drug preparation, may contribute to markedly variable blood drug concentrations both between patients and even within the same patient. Therapeutic management may therefore be facilitated by monitoring blood cyclosporine concentrations. Unfortunately, however, the process of adjusting drug doses based on monitoring cyclosporine blood concentrations is clinically complex, and not necessarily associated with the desired clinical outcome. Currently available methods for TDM include HPLC, a specific monoclonal RIA, and a dimersion cyclosporine immunoassay. HPLC has the advantage that the parent drug can be discriminated from metabolites, although most methods detect only the parent compound. Both RIA and dimension cyclosporine immunoassay, in contrast, measure metabolites as well as the parent drug, and blood cyclosporine concentrations will therefore be higher by a factor of 1.5 to 1.7 compared to the same sample analyzed using HPLC. Although HPLC is considered the gold standard for cyclosporine assays, HPLC is labor intensive and not routinely offered for patient monitoring. TDx and RIA have been the methods most often employed in clinical situations, with the laboratory performing the assay typically providing recommendations regarding ideal target blood drug concentrations. Some laboratories have adjusted target blood concentrations upward to reflect the fact that TDx and RIA results will be approximately double HPLC assay results. Other laboratories have not made this adjustment, with the rationale that the cyclosporine metabolites measured by the TDx and RIA assays may arguably be pharmacologically active and contribute to overall immunosuppressive effects.

Much study has gone into determining the most appropriate sample collection time in patients receiving cyclosporine. In human medicine, trough blood concentrations were the initial basis for adjustment of drug dosages. However, multiple studies in people have since suggested that area under the plasma drug concentration time curve (AUC) or 2 hour peak drug concentrations are preferred. With measurement of peak cyclosporine concentrations requiring only a single
sample, adjusting drug doses to attain target peak drug levels has become the single best blood concentration measurement for use during human organ transplantation. In veterinary medicine, measurement of trough cyclosporine concentrations also prevailed for many years based on initial work done in canine and feline renal transplant studies. Recommendations from laboratories offering TDM have often involved measurement of both peak and trough cyclosporine blood levels, although target peak concentrations have not been well established. Individual laboratory recommendations depended on the target ranges determined by each laboratory as well as the assay used to measure cyclosporine concentrations. Currently, the Auburn University Clinical Pharmacology Laboratory is the only veterinary laboratory routinely offering cyclosporine blood level assays.

Pharmacodynamic assays investigate a drug’s effect on target cells. Several pharmacodynamic biomarkers of the immunosuppressive effects of cyclosporine have been studied in human medicine, including lymphocyte proliferation, calcineurin enzyme activity, lymphocyte surface antigen expression, and intracellular cytokine quantification. Through pharmacodynamic monitoring, human studies have shown individually distinct degrees of calcineurin inhibitor sensitivity in patients. Pharmacodynamic monitoring shows great promise for optimizing cyclosporine therapy and delivering individualized therapy. At Mississippi State University, there are ongoing investigations into the pharmacodynamic evaluation of cyclosporine in dogs. We recently measured activated T cell expression of IL-2, IL-4, and IFN-γ via flow cytometry in dogs administered two different oral cyclosporine dosages. The dogs were first administered a high dose of cyclosporine (10 mg/kg orally twice daily), with doses adjusted upwards as needed to attain a target trough drug concentration greater than 600 ng/mL as measured via HPLC, a dosing protocol known to be sufficiently immunosuppressive for canine organ transplantation. With high dose cyclosporine, activated T cell expression of IL-2 and IFN-γ was significantly suppressed. The dogs were then administered the FDA-approved dose of cyclosporine used to treat canine atopy (5 mg/kg orally once a day), a dose which has been considered to be low enough to avoid predisposing to immunosuppression-associated infection. Even with this low dose of cyclosporine, however, T cell expression of IFN-γ and IL-2 was still markedly suppressed in some dogs. Subsequent studies evaluating activated T cell mRNA IL-2 and IFN-γ expression utilizing molecular methods have demonstrated that results using a quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay are comparable to flow cytometry, and that the technique shows promise as a pharmacodynamic assay in dogs. One advantage of the qRT-PCR assay compared to flow cytometry is that it can be performed on blood samples mailed in by practitioners. Cyclosporine has been shown to have much the same effect on T cell cytokine production in cats as it does in dogs.

Cyclosporine is FDA-approved for the treatment of canine atopic dermatitis and feline allergic skin disease, and has also been used to prevent transplant rejection and to treat sebaceous adenitis, pemphigus foliaceus, anal furunculosis, feline stomatitis, inflammatory bowel disease (IBD), myasthenia gravis, non-infectious inflammatory meningoencephalitis, pure red cell aplasia, immune-mediated hemolytic anemia (IMHA), immune-mediated thrombocytopenia (IMT), and immune-mediated polyarthritis in dogs and cats. Recent pharmacodynamic research evaluating T cell responses to cyclosporine in dogs has confirmed that canine responses are comparable to the response profile that is well recognized in people: that individual responses to cyclosporine are extremely variable from dog-to-dog, both in dogs receiving the same standard oral dose, and in dogs with oral doses adjusted to attain comparable blood levels. Given that a
high degree of variability of individual responsiveness to cyclosporine has been established in dogs, cyclosporine dosing protocols should be tailored to allow for this patient-to-patient variability. In my opinion, recommended dosing protocols in dogs with chronic, non-life-threatening inflammatory skin and gastrointestinal diseases should be quite different from the protocols used in dogs with more acute and life-threatening immune-mediated diseases.

In chronic inflammatory diseases that are typically not immediately life-threatening, such as skin conditions, anal furunculosis, and mild IBD, cyclosporine is often effective at a standard, relatively low starting dose. Cyclosporine therapy is typically delivered long term, with drug doses adjusted upwards if needed ‘to effect’, based predominantly on clinical signs. Most commonly, however, starting doses do not need to be increased and, in the long-term, the cyclosporine dosage is typically tapered to the lowest effective dosage needed to maintain disease remission. Currently recommended starting cyclosporine doses in dogs are 5 mg/kg once daily for most skin diseases and IBD, and 5 mg/kg once to twice daily for anal furunculosis. In cats with skin conditions such as allergic skin disease, eosinophilic granuloma complex and pemphigus foliaceus, a starting cyclosporine dose of around 5 mg/kg daily is recommended. Cyclosporine blood concentrations are usually not necessary for treatment of these conditions, as remission of disease is the main criterion used to decide whether adequate cyclosporine therapy is being delivered. In fact, for many of these conditions, cyclosporine blood concentrations have been shown to have minimal correlation with disease remission, perhaps because the drug is selectively concentrated in tissues such as the skin. Recent pharmacodynamic studies, however, have shown that, even at standard low FDA-approved doses, some dogs can still develop significant suppression of certain T-lymphocyte biomarkers of immunosuppression despite very low trough cyclosporine concentrations. This could explain the phenomenon anecdotally reported by some dermatologists, that individual dogs treated for atopic dermatitis can develop severe secondary infections, although the ‘atopy’ cyclosporine dose was originally not thought to cause clinically significant immunosuppression. Therefore, even in dogs on low cyclosporine doses, clinicians should remain vigilant for potential signs of systemic infection.

In canine patients suffering from more acute and immediately life-threatening diseases such as severe IMHA and IMT, in contrast, cyclosporine must be targeted to attain effective immunosuppression as rapidly as possible. These animals are somewhat comparable to patients that have recently undergone organ transplantation, in that any delay in attaining effective immunosuppression can lead to a disastrous outcome. In these patients, starting cyclosporine at a low dose and adjusting doses upwards ‘to effect’ is not recommended. Attaining effective oral doses as rapidly and accurately as possible is essential for ensuring adequate immunosuppression whilst avoiding overdosage with associated adverse effects and expense. Currently recommended starting cyclosporine doses for life-threatening diseases range from 5 mg/kg to 10 mg/kg twice daily. Subsequent measurement of blood cyclosporine concentrations and/or assessment of activated T cell mRNA IL-2 and IFN-γ expression using qRT-PCR within one week of commencement of treatment, with dose adjustments as needed, are the best methods that are currently routinely available to assess adequacy of therapy, and are strongly recommended in patients with life-threatening diseases.

Side effects are uncommon with cyclosporine therapy in dogs and cats, with the exception of gastrointestinal side effects such as vomiting, diarrhea, anorexia and nausea. Administering the medication frozen and/or with food can reduce gastrointestinal side effects, although there is a
risk that such measures will also alter drug absorption profiles. Uncommonly, cyclosporine can cause an idiosyncratic hepatotoxicity, which does not seem to be dose dependent. Gingival hyperplasia and hypertrichosis have also occasionally been reported with cyclosporine therapy. Chronic cyclosporine therapy may also predispose to neoplasia such as lymphoma. One advantage of cyclosporine as an immunosuppressive agent is that it is not myelosuppressive. Experimentally, oral cyclosporine has been shown to increase some markers of platelet activation in normal dogs, which may be a concern in patients with IMHA, where hypercoagulability and resultant pulmonary thromboembolism can be a major contributor to patient mortality. However, to date, it has not been demonstrated whether this phenomenon is clinically relevant in IMHA patients with naturally occurring disease.

Cyclosporine is an expensive drug, particularly at higher immunosuppressive doses, and clinicians are therefore tempted to explore cheaper forms of the drug. In human medicine, there are many approved human generic microemulsion preparations similar to the Neoral® formulation, and these generic preparations have been shown to have therapeutic equivalency in people. Studies investigating the pharmacokinetic properties of these generic preparations in dogs have not been performed, and it is not safe to assume that a generic formulation is therapeutically equivalent to the approved canine product (Atopica®). Clinically, there appears to be marked variability seen in individual dogs in the oral bioavailability of these generic products. Use of generic products may therefore have the potential place our patients at risk of either therapeutic failure or toxicity. The proprietary human microemulsified cyclosporine product, Neoral®, currently costs around $2 for a 25 mg capsule and $6 for a 100 mg capsule, while the generic equivalent equivalents cost around $1 and $2 for the 25 mg and 100 mg capsules accordingly. The veterinary product, Atopica®, tends to be priced comparably to the human proprietary products, but has the advantage of being FDA-approved and available in a range of capsule sizes that are convenient for dosing accuracy in our small animal patients (10 mg, 25 mg, 50 mg and 100 mg), as well as a 100 mg/ml oral suspension. Unfortunately, transdermal cyclosporine has been shown to be inadequately absorbed in cats.

**Leflunomide**

Leflunomide is an isoxazol derivative immunosuppressive drug that was developed within the past two decades, initially for treatment of rheumatoid arthritis and prevention of transplant rejection. Leflunomide is a prodrug for its primary active malononitriloamide metabolite, A77 1726 (also known as teriflunomide). Malononitriloamides reversibly inhibit the mitochondrial enzyme dihydroorotate dehydrogenase, a key enzyme in pyrimidine synthesis, with resultant inhibition of the pyrimidine ribonucleotide uridine monophosphate (rUMP), and decreased DNA and RNA synthesis and G1 cell cycle arrest. Leflunomide inhibits B and T cell function, suppresses antibody production and has anti-inflammatory effects, possibly via inhibition of de novo pyrimidine biosynthesis and cytokine-associated and IL-2-stimulated tyrosine kinase activity.

Prior to commercial development, leflunomide was made available for small animal transplant research to Dr. Clare Gregory’s group at the University of California, Davis. Because of the drug’s availability to this group, a small number of canine patients with refractory naturally-occurring inflammatory and immune-mediated diseases such as immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, non-infectious inflammatory meningoencephalitis,
systemic histiocytosis, immune-mediated polymyositis, immune-mediated polyarthritis, and pemphigus foliaceous were also treated, typically with promising success rates. Unfortunately, when these initial promising results were reported at the ACVIM Forum and in the veterinary literature in the late 1990s, the drug was not commercially available. When leflunomide did become available, as the proprietary product Arava®, the drug was so prohibitively expensive that its use was very limited in small animal clinical studies. Even after the generic equivalent was approved in 2005, leflunomide remained expensive for several more years. Only recently did the generic drug become more affordable and, as a result, anecdotal and preliminary reports of leflunomide’s use in small animal patients are beginning to surface. There are therefore currently very few published reports discussing the use of leflunomide in dogs and cats. Recently, a case series describing the use of leflunomide in 14 dogs with immune-mediated polyarthritis reported a high response rate with minimal side effects.

One of the most promising features of leflunomide in dogs is that it appears to be very well tolerated although if, as anticipated, the drug attains more common usage, it is likely that less frequent but more serious side effects will be recognized. The most common side effect observed with leflunomide use in dogs is occasional inappetence, lethargy and vomiting. Serious side effects occasionally reported in people, and thus with the potential to appear in our veterinary population with more common usage, include myelosuppression, cutaneous drug reactions and hepatotoxicity. In humans, traces of the active metabolite teriflunomide can persist for months or even years after drug discontinuation, and in the instance of severe drug reactions, cholestyramine or activated charcoal is needed to rapidly reduce drug levels. In dogs, the terminal half-life of teriflunomide is much shorter than in humans, so the potential for persistent side effects is probably significantly less. Complete blood counts and serum biochemistry (especially ALT) should be regularly monitored in small animal patients on leflunomide.

The initial recommended starting oral dose for leflunomide in dogs is 2-4 mg/kg daily, with doses adjusted to attain a plasma trough A77 1726 level of 20 µg/ml within a few weeks of commencing therapy. For cats with immune-mediated polyarthritis, a leflunomide dose 10 mg (total dose) orally, once daily, in combination with methotrexate, has been suggested, with dose reductions to effect. Measurement of leflunomide levels is available through the Auburn University Veterinary Clinical Pharmacology Laboratory. One advantage of leflunomide is that it comes in tablet sizes (10 mg and 20 mg) that are convenient for dosing our smaller patients. Leflunomide as the proprietary product Arava® currently costs around $40 for a 10 mg tablet and, interestingly, $40 for a 20 mg tablet, although it is rumored to soon be discontinued. The generic leflunomide equivalent is currently priced at around $1 for a 10 mg tablet and $1.50 for a 20 mg tablet. Leflunomide generics, as with many commercially available generic problems, have an ‘AB’ rating by the FDA, meaning that the generic is ‘equivalent’ to Arava®. However, since ‘equivalence’ is often determined by pharmacokinetic data in healthy individuals, an AB rating does not guarantee identical performance in clinical patients.

**Mycophenolate**

Mycophenolate mofetil is the synthesized prodrug form of mycophenolic acid, a selective and reversible inhibitor of inosine monophosphate dehydrogenase, an enzyme that controls the rate of synthesis of guanine monophosphate in the *de novo* pathway of purine synthesis. Mycophenolate mofetil is a fermentation product derived from fungi in the *Penicillium* group.
Mycophenolic acid inhibits B and T cell proliferation, and decreases antibody production. Mycophenolate mofetil is primarily used in human medicine for prevention of rejection of transplanted organs, although it also used to treat immune-mediated diseases such as systemic lupus erythematosus, immune-mediated hemolytic anemia (IMHA), immune-mediated thrombocytopenia and pemphigus vulgaris. Mycophenolate mofetil is often used in the place of azathioprine in human medicine and, since they have similar mechanisms of action, the two drugs should probably not be used together.

The original proprietary mycophenolate mofetil product, CellCept®, and the closely related mycophenolate sodium product, Myfortic®, were expensive, and as a result the products only achieved limited usage in small animal medicine. However, recently, the availability of much cheaper generic alternatives has led to a greatly increased usage of mycophenolate mofetil in small animal patients. A single 250 mg CellCept® capsule currently costs around $7, whereas the equivalent generic 250 mg capsule costs less than 50c. An oral suspension version of mycophenolate mofetil (200 mg/ml) is available for more convenient dosing in smaller patients. Successful usage of mycophenolate mofetil in a small animal patient with naturally-occurring disease was first described in a dog with acquired myasthenia gravis. Much of the subsequent anecdotal usage of mycophenolate mofetil for a variety of different immune-mediated diseases was similar to the dosing reported in this original paper. Mycophenolate mofetil is also available in an injectable form, and the intravenous use of the drug has been described during the successful initial stabilization of three dogs with acquired myasthenia gravis that could not tolerate oral medications. Ironically, a more recent case report of 15 dogs with acquired myasthenia gravis treated with mycophenolate mofetil reported that the drug was ineffective at attaining clinical remission.

A recommended starting dose for mycophenolate mofetil in dogs is 10-20 mg/kg once daily or divided twice daily, although occasionally gastrointestinal signs (particularly vomiting and diarrhea) at the higher end of the dose rate will necessitate dose reductions. Mycophenolate mofetil appears to have variable oral bioavailability in dogs, so variability in response to therapy should probably be expected. A pharmacodynamic study in dogs measuring inosine monophosphate dehydrogenase enzyme activity suggested that mycophenolate mofetil would best be dosed three times daily, but this recommendation has not entered common usage. Mycophenolate mofetil has not been used widely enough in veterinary medicine to establish the frequency of serious side effects but, in people, gastrointestinal signs and, less commonly, marked myelosuppression and a rare and fatal neurologic disease (progressive multifocal leukoencephalopathy) have been reported. Based on the human side effect profile, complete blood counts should probably be regularly monitored in dogs receiving mycophenolate mofetil. In humans, gastrointestinal side effects can be reduced by replacing mycophenolate mofetil with mycophenolate sodium. Mycophenolic acid in humans is primarily excreted conjugated to glucuronide and, since cats lack the glucuronyl transferases responsible for glucuronidation of drugs such as mycophenolate mofetil, the drug should probably used with caution, if at all, in this species, although the use of mycophenolate mofetil has been described at a dose rate of 10 mg/kg twice daily, with no obvious side effects, in two cats with IMHA.
One ‘side effect’ that is common to all immunosuppressive agents, both established and new, is that they can cause significant immunosuppression. This is highly desirable when treating severe life-threatening diseases, but comes with the significant associated risk that immunosuppression also predisposes to infection. Severe infection and even, occasionally, infection-associated deaths have been associated with most of the immunosuppressive agents used in dogs and cats. This is especially true when high doses of potent drugs are used, or when multiple drugs are used in combination, such as the kinds of protocols that are used to prevent transplant rejection. As well as bacterial infections, immunosuppressed patients can develop all kinds of unusual infections, including toxoplasmosis, mycobacteriosis, nocardiosis, and generalized demodecosis.

Although the risk of infection is always going to be present when immunosuppressive agents are used, a few general guidelines can help to reduce this risk:

- Avoid using powerful immunosuppressive therapy to treat minor diseases that are not life-threatening, and instead save the ‘big guns’ for more severe illnesses.
- Use the lowest effective drug doses that are possible.
- Avoid using combinations of multiple different immunosuppressive agents unless absolutely necessary.
- Screen patients very carefully for underlying infectious disease before commencing immunosuppressive therapy, especially when infection can mimic immune-mediated disease (*Babesia gibsoni* masquerading as IMHA, for example).
- Watch patients on immunosuppressive therapy closely for signs of new infection.