Update on Immununosuppressive Therapies for Dogs and Cats

Katrina R. Viviano, DVM, PhD

INTRODUCTION

The body’s immune system is essential for protecting it from a variety of pathogens and other external insults. It is through the complex interactions between the cells and mediators of both the innate and adaptive immune systems that the immune system is tightly regulated and homeostasis is established. Autoimmune diseases arise from dysregulation of either the innate or adaptive immune system or both. The complexity of immune dysregulation is not well understood but is likely multifactorial, including inherent genetic factors and/or environmental triggers (ie, infectious agents, drugs, vaccines, or neoplasia). In some patients, the immune system may be appropriately or inappropriately triggered, subsequently leading to lymphocyte dysfunction, for example, failure of lymphocyte selection and/or the generation of antibodies or T cells directed toward self-antigens. The inappropriately stimulated immune system produces a marked local or systemic inflammatory response leading to tissue destruction and clinical disease.

KEYWORDS

- Glucocorticoids
- Cyclosporine
- Azathioprine
- Chlorambucil
- Vincristine
- Human immunoglobulin
- Mycophenolate
- Leflunomide

KEY POINTS

- Understand mechanisms of action, adverse effects, and the clinical limitations of the common drugs (glucocorticoids [GCs], cyclosporine, azathioprine, and chlorambucil) used in the long-term management of immune-mediated diseases.
- Appreciate the clinical situations in which the use of human intravenous immunoglobulin (hIVIG) versus vincristine is most appropriate.
- Recognize the advantages and disadvantages of using mycophenolate and leflunomide in the treatment of immune-mediated disease in dogs and cats.
Some of the more common systemic inflammatory diseases with an autoimmune cause in dogs and cats include protein-losing enteropathy/inflammatory bowel disease, immune-mediated hemolytic anemia, immune-mediated thrombocytopenia (IMT), immune-mediated polyarthritis, and feline asthma. Despite the varying and incompletely understood pathogenic mechanisms or triggers among this group of diseases, immune dysfunction is central to tissue injury and the rationale for use of immunomodulatory therapies. The focus of this article is to provide an update on some of the more common immunosuppressive therapies used in small animal veterinary medicine. Few of these immunosuppressive drugs or protocols are well studied in veterinary species but their use has been adapted or extrapolated from human medicine in the treatment of autoimmune diseases or in the management of organ transplantation. The goals of therapy are to induce disease remission through the inhibition of inflammation and the modulation of lymphocyte function.

FIRST-LINE AND SECOND-LINE IMMUNOSUPPRESSIVE DRUGS

**Glucocorticoids**

**Proposed mechanism**

Glucocorticoids (GCs) affect most, if not all, cells of the body through their binding to the intracellular cytoplasmic GC receptor. Once the GC-receptor complex is translocated to the nucleus, it binds DNA GC response elements influencing gene transcription. The cellular effects of GCs are considered dose dependent. At antiinflammatory doses, GCs inhibit phospholipase A2 and the release of proinflammatory cytokines as well as stabilize granulocyte cell membranes. At immunosuppressive doses, GCs target macrophage function by down regulating Fc receptor expression, decreasing responsiveness to antibody-sensitized cell and decreasing antigen processing. GCs suppress T-cell function and induce apoptosis of T cells and with chronic use B-cell antibody production may be inhibited in some patients.

**Adverse effects/drug interactions**

The wide cellular/tissue distribution of the GC receptor makes significant systemic effects unavoidable. Adverse effects include iatrogenic hyperadrenocorticism, adrenal gland suppression, gastrointestinal ulceration, insulin resistance and secondary diabetes mellitus, muscle catabolism, delayed wound healing, opportunistic infections, and behavior changes. Clinical signs of hyperadrenocorticism are more common in dogs and include polydipsia, polyuria, polyphagia, weight gain, and increased panting. In addition, some dogs experience elevations in serum alkaline phosphatase activity secondary to the induction of the steroid-induced isoenzyme. The coadministration of GC with nonsteroidal antiinflammatory drugs is contraindicated due to the significant risk of gastrointestinal ulceration or perforation.

**Clinical use**

Despite the long list of clinically significant adverse effects associated with either short-duration high-dose or chronic low-dose GC therapy, GCs remain the mainstay or first-line therapy in the treatment of inflammatory and autoimmune diseases in dogs and cats (e.g. immune-mediated anemia, IMT, immune-mediated polyarthritis, inflammatory bowel disease, and feline asthma). Advantages of GCs are their systemic impact on both innate and acquired immunity and their relative rapid onset of action, thereby maintaining their role in the acute management of inflammatory/immune-mediated diseases. The goal of therapy is to achieve clinical remission and slowly taper the dose of GCs to the lowest dose that controls the inflammatory or immune-mediated disease targeted. On a case-by-case basis, GCs may be used in combination
with other immunosuppressive agents in the treatment of inflammatory or immune-mediated diseases in dogs and cats, especially in patients that are nonresponsive to GCs alone or present with severe life-threatening disease.

**Pharmacokinetics/pharmaceutics**

Available GCs vary in their potency, route of administration, and duration of action (Table 1). The most common intermediate-acting systemic GCs used in veterinary medicine include prednisone/prednisolone. Prednisone is a prodrug that is metabolized to its active form prednisolone. Cats are reported to achieve higher plasma concentrations (4–5 times higher area under the curve) when administered oral prednisolone versus prednisone, suggesting cats either have lower prednisone absorption and/or decreased prednisone conversion to prednisolone.

Alternative forms of GCs should be considered in specific patient populations. In patients with severe malabsorption, injectable dexamethasone sodium phosphate may provide improved bioavailability as well as clinical response. Also, dexamethasone lacks mineralocorticoid activity minimizing sodium and water retention. This may be clinically significant in treating patients with underlying cardiovascular disease or diseases associated with fluid retention (e.g. hypoalbuminemia or portal hypertension). The potency of dexamethasone is 4 to 10 times that of prednisone; therefore, a dose reduction is necessary when prescribing dexamethasone.

In some patients, locally delivered GCs may be advantageous. Budesonide is an oral, locally active, high-potency GC that is formulated to exploit the pH differential between the proximal and distal small intestine, targeting budesonide’s action to the distal intestinal tract. Budesonide is absorbed at the level of the enterocyte delivered by the portal system to the liver, where 80% to 90% of the absorbed budesonide undergoes first pass metabolism minimizing its systemic bioavailability. Some systemic absorption occurs, as evidenced by a blunted ACTH (cosyntropin) stimulation test in dogs treated with budesonide at 3 mg/m² for 30 days. Budesonide is used in management of Crohn disease in humans and inflammatory bowel disease in dogs. In dogs and cats with inflammatory respiratory disease, locally delivered fluticasone can be effectively inhaled to control clinical signs in patients with severe respiratory disease.

<table>
<thead>
<tr>
<th>Type</th>
<th>Potency</th>
<th>Route of Administration</th>
<th>Site of Action</th>
<th>Biologic Half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone/cortisol</td>
<td>1</td>
<td>IV</td>
<td>Systemic</td>
<td>8–12 h</td>
</tr>
<tr>
<td>Prednisone</td>
<td>4</td>
<td>PO</td>
<td>Systemic</td>
<td>12–36 h</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>4</td>
<td>PO</td>
<td>Systemic</td>
<td>12–36 h</td>
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<tr>
<td>Dexamethasone</td>
<td>30</td>
<td>PO</td>
<td>Systemic</td>
<td>&gt;48 h</td>
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<tr>
<td></td>
<td></td>
<td>IV (SP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Budesonide</td>
<td>60</td>
<td>PO</td>
<td>Intestinal Liver</td>
<td>t₁/₂ = 2 h (canine)</td>
</tr>
<tr>
<td>Fluticasone</td>
<td>540</td>
<td>Inhaled</td>
<td>Lungs</td>
<td>t₁/₂ = 8 h (human)</td>
</tr>
</tbody>
</table>

Abbreviation: dex SP = dexamethasone sodium phosphate.

GC-responsive inflammatory airway disease, which minimizes the adverse systemic side effects of GC therapy.\textsuperscript{22–24}

**Cyclosporine**

**Proposed mechanism**
Cyclosporine is a polypeptide consisting of 11 amino acids, derived from a Norwegian soil fungus, *Tolypocladium inflatum*.\textsuperscript{25} Cyclosporine inhibits the activation of T cells through the intracellular target calcineurin.\textsuperscript{26,27} T lymphocytes express cyclophilin in high concentrations. Cyclophilin is an immunophilin or highly conserved protein that acts as a protein-folding enzyme (or proline isomerase).

Cyclosporine binds to cytoplasmic cyclophilin forming a cyclosporine-cyclophilin complex. The cyclosporine-cyclophilin complex binds and blocks the function of calcineurin, a serine/threonine phosphatase that is activated by increased intracellular calcium concentrations after T-cell receptor activation. Calcineurin functions to dephosphorylate the nuclear factor of activated T cells, enabling it to translocate into the nucleus, bind the nuclear transcription factor, activator protein 1, and induce transcription of genes for T-cell activation. The cyclosporine-cyclophilin complex prevents the dephosphorylation of nuclear factor of activated T cells, decreasing the expression of interleukin (IL)-2 and other cytokines, preventing further T-cell activation. Decreased IL-2 concentrations attenuate clonal proliferation of T lymphocytes and B lymphocytes. Cyclosporine also decreases the production of IL-3, IL-4, and tissue necrosis factor alpha, altering the function of granulocytes, macrophages, natural killer cells, eosinophils, and mast cells. In small animal veterinary species, cyclosporine is reported to decrease lymphocyte cytokine production in feline lymphocytes in vitro\textsuperscript{28} and in canine lymphocytes in vitro and in vivo.\textsuperscript{29,30}

**Adverse effects/drug interactions**
Historically, cyclosporine has been used for its GC-sparing effects, its rapid onset of immunosuppression, and the potential for less systemic adverse effects. Mild gastrointestinal upset after oral cyclosporine administration is the most common side effect reported in dogs and cats. It is often transient or responsive to dose reduction and does not require drug discontinuation. In some cases, more severe systemic side effects have been reported in cats and dogs, which require the discontinuation of cyclosporine to include gingival hyperplasia, opportunistic infections, hepatotoxicity, and lymphoproliferative disorders.\textsuperscript{31,32} Parenteral cyclosporine administration in a cat has been reported to cause anaphylaxis.\textsuperscript{33} Another potential complication associated with cyclosporine administration in dogs is the potential for thromboembolic complications.\textsuperscript{34} Similar thromboembolic complications have been reported in human transplant patients associated with cyclosporine administration.\textsuperscript{35–37}

Cyclosporine is a substrate of cytochrome P450, family 3, subfamily A (CYP3A), and drug interactions associated with cyclosporine administration are reported due to either the inhibition or induction of the cytochrome P450 enzyme system. Cyclosporine is also a substrate of the drug transporter, P-glycoprotein, which also influences its disposition and leads to potential drug interactions. Clinically relevant drug interactions reported in dogs or cats as a result of the decreased cyclosporine metabolism and increased cyclosporine blood levels include its coadministration with azole antifungals,\textsuperscript{38–41} clarithromycin,\textsuperscript{42} and grapefruit juice.\textsuperscript{43,44} The coadministration of cyclosporine with ketoconazole is often used therapeutically to decrease the dose and cost of cyclosporine while maintaining therapeutic blood levels.\textsuperscript{40,41} The presumed mechanism of this exploited drug interaction is via inhibition of CYP3A and/or P-glycoprotein efflux.\textsuperscript{45}
Clinical use
The initial use of cyclosporine in human and veterinary medicine was in the management of transplant recipients, but more recently cyclosporine has been used in the treatment of inflammatory and immune-mediated diseases.

Specifically in veterinary medicine, cyclosporine is considered first-line therapy for perianal fistulas, keratoconjunctivitis sicca or dry eye, and in some patients with atopic dermatitis. The use of cyclosporine in the treatment of other inflammatory or immune-mediated diseases is as a second-line immunosuppressive agent based primarily on published retrospective case series. Examples include its use to treat feline inflammatory bowel disease, hemolytic anemia, pure red cell aplasia, and thrombocytopenia.

Pharmacokinetics/pharmaceutics
The pharmacokinetics of cyclosporine is significantly impacted by the formulation (oil-based vs microemulsion), the species being treated, and the patient’s intrinsic liver function/dysfunction or concurrently administered medications. Despite the improvement in gastrointestinal absorption and intrapatient and interpatient variability in blood concentrations associated with the routine use of the microemulsion versus the oil-based formulation of cyclosporine, the disposition of cyclosporine remains variable. In some patients, therapeutic drug monitoring is indicated. Knowing a patient’s history and concurrent medications, the cyclosporine formulation administered, and the assay method used for monitoring blood levels is essential. For example, the assays used to quantify cyclosporine blood levels vary depending on the compartment analyzed (plasma vs whole blood) and whether an immunoassay or a high-performance liquid chromatography method is used.

In veterinary medicine, the targeted blood levels of cyclosporine necessary for the effective treatment of immune-mediated diseases are not as clearly established as they are in transplant medicine. A suggested therapeutic goal for cyclosporine in the treatment of immune-mediated cytopenias in humans is a whole-blood trough cyclosporine concentration between 150 ng/mL to 250 ng/mL for a maximum of 3 to 4 months followed by maintenance therapy with the minimum dosage to maintain remission. No clinical therapeutic data are available for cyclosporine in the treatment of immune-mediated diseases in dogs and cats. Trough whole-blood cyclosporine levels between 400 ng/mL and 600 ng/mL are routinely used as the therapeutic target in veterinary medicine for efficacy and safety, which has been extrapolated from transplant patients. Trough levels, however, often do not reliably predict clinical response. Research continues to explore immunosuppressive markers that parallel cyclosporine’s clinical response, including the use of drug exposure, T-cell cytokine expression, and lymphocyte-specific proliferation.

Tacrolimus is related to cyclosporine by its similar mechanism of action and immunosuppression. Tacrolimus also binds a cytoplasmic immunophilin, FKBP12, functioning as a potent immunosuppressive agent but has limited use in cats and dogs due to its severe systemic side effects. The clinical use of tacrolimus in veterinary medicine is as a topical therapy for perianal fistulas, keratoconjunctivitis sicca, or dermatitis.

Azathioprine
Proposed mechanism
Azathioprine is a thiopurine that is a prodrug of 6-mercaptopurine. In the liver and other peripheral tissues (e.g. erythrocytes), 6-mercaptopurine is enzymatically oxidized to inactive metabolites 6-thiouric acid via xanthine oxidase or methylated
via thiopture methyltransferase (TPMT) to 6-methylmercaptopurine. The 6-thioguanine nucleotides (6-TGNs) are generated via hypoxanthine phosphoribosyl transferase, the active metabolites responsible for both the therapeutic and cytotoxic effects of azathioprine. 6-TGNs compete with endogenous purines for incorporation into RNA and DNA, creating nonfunctional DNA and RNA and disrupting DNA and RNA synthesis and mitosis. Azathioprine targets cell-mediated immunity, specifically lymphocytes, due to their lack of a salvage pathway for purine biosynthesis. Through its inhibition of de novo purine synthesis azathioprine interferes with lymphocyte proliferation, reduces lymphocyte numbers, and decreases T-cell–dependent antibody synthesis.

**Adverse effects/drug interactions**

The most common adverse effects attributed to azathioprine administration and subsequent drug withdrawal in humans and dogs include myelosuppression and gastrointestinal upset (vomiting and diarrhea). Azathioprine myelosuppression is a delayed response, occurring after 1 to 2 weeks of therapy, which is reversible after drug withdrawal. One study reports a 13% prevalence of myelosuppression (dose-dependent neutropenia and thrombocytopenia) in dogs with immune-mediated hemolytic anemia (IMHA) treated with azathioprine and prednisone for 3 months. Other less-common adverse effects include hepatic necrosis and pancreatitis.

Due to the risk of hepatitis and hepatic necrosis prior to and after the initiation of azathioprine therapy, monitoring liver enzymes is recommended. In humans, hepatotoxicity has been correlated with increased erythrocyte 6-methylmercaptopurine concentrations. In rats, liver necrosis associated with azathioprine administration results in oxidative damage, glutathione depletion, and marked increases alanine aminotransaminase activity.

**Clinical use**

In humans, azathioprine is used for the treatment of immune-mediated disease and organ transplant medicine. Its use in treating immune-mediated diseases in dogs is in part due to its GC-sparing effect, which enables sustained disease remission while tapering or after GC withdrawal. Its effectiveness in the treatment of acute immune-mediated illness is limited based on its delayed efficacy of days to weeks. Few controlled studies have been published evaluating the use of azathioprine in treating immune-mediated disease in dogs; therefore, its use is reliant on published retrospective studies reporting its use in the treatment of IMHA.

**Pharmacokinetics/pharmaceutics**

In humans, TPMT activity is variable and correlates with clinical outcomes, including therapeutic efficacy and toxicity. Due to a genetic polymorphism, some individuals have increased or decreased TMPT activity. Decreased TMPT activity is associated with an increased risk of azathioprine-induced myelosuppression due to increased substrate availability for HRPT and the generation of cytotoxic 6-TGNs. A 9-fold difference in TPMT activity has been reported in dogs, with lower TMPT activity in giant schnauzers and higher TMPT activity in Alaskan malamutes. Compared with dogs or humans, cats have decreased TMPT activity, which increases their risk of toxicity. The use of azathioprine in cats is generally avoided and, if used, a significant dose reduction is recommended.

Allopurinol, a xanthine oxidase inhibitor, results in a significant increase in 6-TGN concentrations, increasing the risk of azathioprine toxicity (ie, myelosuppression). In humans, historically concurrent therapy of azathioprine with allopurinol was considered contraindicated or minimally required significant azathioprine dose reduction to
avoid significant adverse drug-drug interactions. In human patients with high TPMT activity and inflammatory bowel disease nonresponsive to azathioprine therapy, the concurrent administration of allopurinol with azathioprine has been exploited to increase the concentration of 6-TGNs and induce disease remission.

**Chlorambucil**

**Proposed mechanism**
Chlorambucil is a nitrogen mustard derivative and prodrug that is converted in the liver to its active metabolite, phenylacetic acid. It is a cell-cycle nonspecific, cytotoxic, alkylating agent capable of cross-linking DNA. Chlorambucil targets B cells and is considered a slow-acting immunosuppressive agent that may require 2 weeks to reach therapeutic efficacy.

**Adverse effects/drug interactions**
Relative to azathioprine in cats, chlorambucil has less adverse side effects. Cytotoxic myelosuppression and gastrointestinal toxicity are associated with chlorambucil administration. Myelosuppression is considered mild and generally occurs 7 to 14 days after the start of therapy. Neurotoxicity (ie, reversible myoclonus) has been reported in a cat in association with a chlorambucil overdose.

**Clinical use**
Prospective clinical studies evaluating the use of chlorambucil as an immunosuppressive agent are lacking. The majority of published studies in cats focus on the use of chlorambucil as a chemotherapeutic agent in the treatment of lymphoma. Despite the paucity of data, chlorambucil is most often used as the cytotoxic drug of choice in cats. Its role as a second-line immunosuppressive therapy in cats has been used in the treatment of inflammatory bowel disease that is either severe or poorly responsive to prednisone/prednisolone therapy. In a recent case series of cats with IMT, chlorambucil was successful as a second-tier drug in one cat that failed to achieve remission with prednisone alone.

**ADJUNCTIVE IMMUNOMODULATORY THERAPIES**

In veterinary medicine, vincristine and human intravenous immunoglobulin (hIVIg) are considered adjunctive therapies to standard immunosuppressive protocols in the acute management of patients with immune-mediated diseases. Advantages include their potential for initial disease stabilization due to the potential for a relative fast therapeutic response as well as the low likelihood of adverse effects. Based on the limited objective published studies in veterinary medicine, the patient populations that may most likely benefit for either vincristine or IVIg are dogs with severe IMT. These include either dogs that failed to respond to standard immunosuppressive protocols or dogs in which their initial clinical presentation is severe, including a substantial risk of a fatal hemorrhage. As in humans with immune thrombocytopenic purpura, these adjunctive agents are not typically useful as a single-agent therapy.

**Vincristine**

**Proposed mechanism**
Vincristine is an alkaloid derived from the periwinkle plant. Several mechanisms have been proposed by which vincristine increases platelet counts, including stimulating megakaryocyte fragmentation and impairing microtubule assembly within macrophages, interfering with their ability to phagocytize optimized platelet. In humans, as
a single-agent therapy, vincristine only increases platelet counts in a subset of refractory patients with chronic thrombocytopenia.

**Adverse effects/drug interactions**
In dogs, the vincristine dose, 0.02 mg/kg, reported as used in the treatment of IMT, has not been associated with a clinically significant myelosuppression. Other potential side effects of vincristine therapy include gastrointestinal upset (anorexia, vomiting, or diarrhea) or peripheral neuropathy (rare). Extreme caution should be used during the administration of vincristine to avoid extravasation and perivascular sloughing.

**Clinical use**
Vincristine’s usefulness may be as emergent therapy in patients with IMT that are actively bleeding and transfusion dependent with evidence of megakaryocyte hyperplasia. Its use as an adjunctive therapy is to either initially increase platelet counts to enable initial patient stabilization while waiting for a clinical response from standard immune-mediated therapies or as a salvage therapy to increase platelet counts in patients with refractory immune mediated thrombocytopenia.

In 24 dogs with severe IMT, Rozanski and colleagues reported dogs treated with prednisone and vincristine experienced an increase in their platelet count to greater than 40,000/µL in a mean of 4.9 versus 6.8 days and a shorter duration of hospitalization a mean of 5.4 versus 7.3 days than dogs treated with prednisone alone. None of the dogs treated with vincristine experienced any adverse effects, including no increased risk of bleeding suggestive of altered platelet function after vincristine administration. The effect of vincristine on platelet function in dogs with IMT has not been evaluated. In healthy dogs, however, the administration of vincristine is reported to stimulate thrombopoiesis and has no adverse affect in vivo platelet function. This is in contrast to dogs with lymphoma in which the administration of vincristine results in abnormal platelet aggregation.

**Human Intravenous Immunoglobulin**

**Proposed mechanism**
hIVIg is a purified product of pooled human plasma from multiple healthy donors. Approximately 90% of hIVIg is purified IgG with trace concentrations of IgA, IgM, CD4, CD8, and HLA molecules. In the treatment of immune-mediated diseases, hIVIg is used for its ability to regulate the immune system, inhibit phagocytosis, and decrease tissue damage. The immunomodulatory actions of hIVIg are not well understood but the efficacy of hIVIg therapy in the treatment of immune-mediated diseases has been attributed to multiple mechanisms. Some of the dominant mechanisms of action of hIVIg include Fc receptor blockade, autoantibody elimination, cytokine modulation, complement inhibition, and Fas–Fas ligand blockade.

**Adverse effects/drug interactions**
The most anticipated adverse effect of hIVIg in veterinary patients is the risk of an acute hypersensitivity reaction due to the infusion of human-derived foreign proteins into canine or feline species. Other adverse effects reported in human patients treated with hIVIg include hypotension, thromboembolism, renal insufficiency, and dose-dependent aseptic meningitis. In human patients, a decrease in acute hypersensitivity reactions and other adverse effects are associated with the use of hIVIg products that have been processed to remove the IgG aggregates or contain low IVIg concentrations and with the use of non–sucrose-containing products as well as the use of slow infusion rates.
No significant adverse effects have been reported in association with hIVIG administration in clinically ill dogs and cats.\textsuperscript{95,116–120} Of the reported studies available, however, different doses and hIVIg products were used and, in some of the canine studies, diphenhydramine was administered prior to the infusion of hIVIg,\textsuperscript{95,121,122} making the true assessment of the adverse effects of hIVIg difficult. An experimental study in healthy beagles supports an inflammatory/hypercoagulable state after the administration of hIVIg.\textsuperscript{123} Conclusive clinical studies in dogs, specifically in dogs with concurrent prothrombotic conditions, are lacking.\textsuperscript{124} In addition, limited data are available on the safety of repeated or multiple hIVIg transfusion in dogs.\textsuperscript{116–118,120,125} and more studies are necessary to assess the risk before repeated hIVIg infusions can be recommended.

**Clinical use**
The use of hIVIg in humans initially began in the treatment of immunodeficiency disorders but now includes a wide range of immune-mediated and inflammatory disorders despite the limited number of Food and Drug Administration–approved conditions.\textsuperscript{126–130} The 7 approved conditions for hIVIg use in human patients included Kawasaki disease, bone marrow transplantation, idiopathic thrombocytopenic purpura, chronic B-cell lymphocytic leukemia, pediatric HIV, chronic inflammatory demyelinating polyneuropathy, and primary immunoglobulin deficiency.\textsuperscript{128} Canine lymphocytes and monocytes have been shown to be effectively bound by.\textsuperscript{131} The first reports of the clinical use of hIVIg in veterinary medicine were in dogs with immune-mediated hemolytic anemia.\textsuperscript{119,132} The use of hIVIg in veterinary medicine has since expanded to its use as an adjunctive therapy in a variety of immune-mediated diseases, including hemolytic anemia,\textsuperscript{56,124} thrombocytopenia,\textsuperscript{95,121} immune-mediated cutaneous diseases,\textsuperscript{116,118,120,125} and sudden acquired retinal degeneration syndrome.\textsuperscript{117}

The potential advantage of hIVIg is the ability of IgG to quickly block the Fc receptor and provide initial disease stabilization by decreasing immune-mediated destruction and continued tissue injury. This initial patient stabilization provides time for the traditional long-term immunomodulatory therapies to become clinically effective, shortens hospitalization, and decreases the dependence on repeated transfusions and additional supportive care.\textsuperscript{116,118–121,125} No consensus on the use of hIVIg in veterinary patients with immune-mediated diseases has been established because most data are limited to retrospective studies,\textsuperscript{119,121,133} case reports,\textsuperscript{95,116,118,125} and few prospective, randomized, investigator-blinded, placebo-controlled clinical trials.\textsuperscript{122,124} A prospective, randomized, double-blinded, placebo controlled clinical trial has been published supporting the use of hIVIg in dogs with IMT. Bianco and colleagues\textsuperscript{122} treated all dogs with severe IMT with immunosuppressive doses of prednisone, then randomized the dogs to either placebo or a single infusion of hIVIg within 24 hours of the initiation of prednisone therapy. The dogs treated with prednisone and hIVIg had a significant reduction in time to resolution of their thrombocytopenia (mean 3.7 vs 7.5 days; $P = .0180$) and number of days hospitalized (mean 4 vs 8 days; $P = .0270$) compared with dogs treated with prednisone alone. There was no significant difference, however, in the number of blood transfusions, cost, or mortality. Treatment of dogs with severe IMT with prednisone and hIVIg resulted in similar response times and days of hospitalization as reported for canine patients with IMT treated with prednisone and vincristine by Rozanski and colleagues\textsuperscript{96} (Table 2). Further studies are needed to identify if hIVIg has benefits over vincristine in treating dogs with severe IMT.
In a blinded clinical trial, 28 dogs with IMHA were randomized to either treatment with hIVIg versus placebo (0.9% NaCl) as part of their initial drug therapy and disease stabilization. All dogs were treated concurrently with prednisone and low-molecular-weight heparin. There was no difference in days to stabilization of PCV or duration of hospitalization between the 2 treatment groups.

**Pharmacokinetics/pharmaceutics**

A range of hIVIg products is available with varying IgG concentrations, osmolality, and sugar content. The dose and duration of hIVIg infusions in veterinary patients have been extrapolated from its use in human medicine. Published reports in dogs and cats have used doses ranging from 0.25 g/kg to 2.2 g/kg, with infusion durations ranging from 4 hours to 12 hours. The specific hIVIg products used have varied as well. The use of hIVIg products in clinical veterinary medicine may be more a function of its availability to veterinary clinicians and the dose used may be influenced by cost. In many cases, the use of hIVIg may be cost prohibitive in veterinary patients.

**EMERGING IMMUNOSUPPRESSIVE THERAPIES**

**Mycophenolate Mofetil**

**Proposed mechanism**

Mycophenolate mofetil (MMF), the prodrug of mycophenolic acid (MPA), is used in human medicine as an alternative immunosuppressant to azathioprine in transplant medicine and in the treatment of immune-mediated diseases. MPA is a potent, selective, noncompetitive, reversible inhibitor of inosine-5’-monophosphate dehydrogenase (IMPDH), specifically, the type II isoform of IMPDH. The IMPDH type II isoform is more abundant in activated lymphocytes and is 5 times more susceptible to MPA than the type I isoform (expressed in many cell types). IMPDH catalyzes the rate-limiting step of the de novo biosynthesis of guanosine nucleotides, which converts inosine monophosphate to guanosine monophosphate. MPA’s cytotoxicity is selective to lymphocytes via the depletion of guanosine and deoxyguanosine nucleotides. T lymphocytes and B lymphocytes are entirely dependent on the de novo pathway for purine synthesis, differentiation, proliferation, and immunoglobulin production. Other mechanisms that contribute to MMF’s effectiveness in treating inflammatory and immune-mediated diseases are via its suppression of dendritic cell maturation and reduction in monocyte recruitment into the site of inflammation.

<table>
<thead>
<tr>
<th>Study</th>
<th>Prednisone (3 mg/kg/d)</th>
<th>(+) Vincristine (0.02 mg/kg IV)</th>
<th>(+) hIVIG (0.5 g/kg IV)</th>
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</thead>
<tbody>
<tr>
<td>Rozanski et al, 96 2002 (n = 24)</td>
<td>&gt;40 K/uL plt (days) 6.8 ± 4.5</td>
<td>&gt;40 K/uL plt (days) 4.9 ± 1.1</td>
<td>&gt;40 K/uL plt (days) —</td>
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<tr>
<td></td>
<td>ICU (days) 7.3 ± 0.5</td>
<td>ICU (days) 5.4 ± 0.3</td>
<td>ICU (days) 3.7 ± 1.3</td>
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<td>Bianco et al, 122 2009 (n = 18)</td>
<td>&gt;40 K/uL plt (days) 7.8 ± 3.9</td>
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<td></td>
<td>ICU (days) 8.3 ± 0.6</td>
<td>ICU (days) —</td>
<td>ICU (days) 4.2 ± 0.4</td>
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</table>

**Abbreviation**: plt = platelets.

Data reported as mean ± SD.
In addition, MMF may be antifibrotic through its ability to inhibit proliferation of non–immune cells, including smooth muscle, renal tubular, and mesangial cells.\textsuperscript{142}

\textbf{Adverse effects/drug interactions}

Side effects reported in humans treated with MMF include gastrointestinal upset, opportunistic infections, allergic reactions, neutropenia, and lymphoma.\textsuperscript{140,143} Limited information is available about the adverse effects of MMF in dogs and cats. The primary side effects reported in dogs treated with oral MMF are diarrhea and weight loss.\textsuperscript{144,145} Mild allergic reactions have been reported with the administration of parenteral MMF in dogs.\textsuperscript{146}

Drug interactions reported in humans treated with MMF resulting in decreased MMF’s bioavailability may also be clinically relevant in veterinary patients. The concurrent administration with some antibiotics (ie, fluoroquinolones and metronidazole) reduces the enterohepatic circulation of MMF.\textsuperscript{147} If an enteric-coated formulation of oral MMF is not used, the higher gastric pH achieved with proton pump inhibitors reduces the dissolution of MMF and decreases drug exposure.\textsuperscript{148} Cyclosporine decreases MMF exposure via inhibition of the enterohepatic recirculation of MMF due to reduced biliary excretion of the glucuronide metabolite by multidrug resistance protein 2 transporter.\textsuperscript{149} GCs induce the uridine diphosphate glucuronosyltransferase enzyme system, increasing the metabolism of MMF.\textsuperscript{150} Consideration should be given to possible drug-drug interactions in patients nonresponsive to MMF. The concurrent use of MMF with azathioprine is not recommended based on their similar mechanism of action and risk of bone marrow suppression.\textsuperscript{140}

\textbf{Clinical use}

The use of mycophenolate in dogs and cats with refractory inflammatory or immune-mediated diseases continue to emerge in the literature as case reports or case series. Canine diseases reported as responsive to MMF include aplastic anemia\textsuperscript{151} and subepidermal blistering autoimmune skin disease.\textsuperscript{152} Recently, a case report was published describing the successful use of MMF in 2 cats with refractory immune-mediated hemolytic anemia.\textsuperscript{153} A small retrospective case series evaluating dogs with acquired myasthenia gravis treated with mycophenolate and pyridostigmine versus pyridostigmine alone was not supportive of the use of MMF based on no differences in remission rates, time to remission, and survival.\textsuperscript{145}

\textbf{Pharmacokinetics/pharmaceutics}

In veterinary patients MMF is anticipated to have a rapid onset of action (maximal IMPDH inhibition 2–4 hours post oral administration) and acceptable tolerability.\textsuperscript{146,154,155}

\textbf{Leflunomide}

\textbf{Proposed mechanism}

Leflunomide is a synthetic isoxazole derivative that is metabolized to its active metabolite, malononitrilamide, or teriflunomide.\textsuperscript{156–158} Teriflunomide primarily functions as a selective pyrimidine synthesis inhibitor via the reversible inhibition of dihydroorotate dehydrogenase, a mitochondrial enzyme required for de novo pyrimidine biosynthesis. Other possible mechanisms of actions include inhibition of tyrosine kinase activity, which alters cytokine and growth factor receptors associated with tyrosine kinase activity. Leflunomide targets B lymphocytes and T lymphocytes, which lack a pyrimidine salvage pathway. In vitro canine B and T cells were sensitive to the antiproliferative effects of malononitrilamide.\textsuperscript{159} In a dose-dependent manner,
malononitrilamide has been reported to inhibit mitogen-stimulated proliferation of feline lymphocytes in vitro.\textsuperscript{160}

**Adverse effects/drug interactions**

Reported clinical side effects in dogs include lethargy, gastrointestinal upset, and mild bone marrow suppression (leukopenia and thrombocytopenia).\textsuperscript{161,162} Dose-dependent myelosuppression has been reported in dogs treated with leflunomide at dosages greater than or equal to 4 mg/kg once a day.\textsuperscript{163} Anecdotal reports of severe myelosuppression (ie, leukopenia) and in some cases bone marrow necrosis have been associated with leflunomide therapy in dogs. In humans, severe idiosyncratic reactions have been reported with leflunomide therapy, including myelosuppression, hepatotoxicosis, and toxic epidermal necrolysis.\textsuperscript{156,157}

**Clinical use**

Leflunomide has been used in humans for its immunosuppressive and antiproliferative effects in the treatment of rheumatoid arthritis, Crohn disease, and systemic lupus erythematosus and the management of transplant recipients.\textsuperscript{156,157} Gregory and colleagues\textsuperscript{162} initially described the use of use leflunomide as an add-on therapy for a variety of naturally occurring immune mediated/inflammatory disease in dogs, including IMT, immune-mediated hemolytic anemia, systemic histocytosis, nonsuppurative encephalitis/meningomyelitis, immune-mediated polymyositis, immune-mediated polyarthritis, and pemphigus foliaceus. Additional case reports and retrospective studies have been published describing the successful use of leflunomide in companion animal medicine. Leflunomide has been used in the treatment of reactive histocytosis,\textsuperscript{164} and Evans syndrome\textsuperscript{95} in dogs. A retrospective clinical study evaluating leflunomide in the treatment of immune-mediated polyarthritis was recently published;\textsuperscript{161} 8 of the 14 dogs with immune-mediated polyarthritis had resolution of their clinical signs after the introduction of leflunomide. In a group of cats (n = 12) with rheumatoid arthritis, previously nonresponsive to standard therapy, leflunomide was used in conjunction with methotrexate to provide marked clinical improvement in half the cats.\textsuperscript{165}

**SUMMARY**

The treatment of immune-mediated disease in dogs and cats continues to evolve as new therapies are introduced or adapted from human medicine. GCs remain the first-line therapy for many of the immune-mediated or inflammatory diseases of cats and dogs. Therapies incorporating cyclosporine, azathioprine, or chlorambucil are the more common second-line therapies used, but their use is clinician and patient dependent. Often, these second-line therapies are introduced due to a patient’s lack of response or intolerable side effects associated with GC therapy or may be introduced early in the disease process due to a patient’s severe life-threatening clinical presentation. Table 3 summarizes and compares the more common non-GC immunosuppressive drugs used in dogs and cats. The introduction of adjunctive therapies (vincristine and hIVIG) aid in the initial disease stabilization and may decrease the duration of hospitalization and need for prolonged intensive supportive care. In dogs, the benefits of adjunctive therapies are best documented for idiopathic IMT.

For those refractory patients or patients with intolerable side effects associated with the standard immunosuppressive therapies, veterinary medicine continues to borrow alternative immunosuppressive agents from the experience of human medicine as new drugs continue to emerge. As emerging immunosuppressive therapeutics for veterinary patients are initially adopted, knowledge of their therapeutic efficacy and
<table>
<thead>
<tr>
<th>Drug</th>
<th>MOA</th>
<th>Dosage(s)</th>
<th>Indications</th>
<th>Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporine</td>
<td>Calcineurin inhibitor</td>
<td>2.5 mg/kg PO q 12 h (with ketoconazole) or 4 mg/kg PO q 12 h</td>
<td>Canine—perianal fistulas[^41,48]</td>
<td>GI upset, gingival hyperplasia, hepatotoxicity, opportunistic infections, anaphylaxis (IV)</td>
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<tr>
<td></td>
<td></td>
<td>5 mg/kg PO q 24 h</td>
<td></td>
<td>Potential drug interactions—ketoconazole, clarithromycin, grapefruit juice</td>
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<tr>
<td></td>
<td></td>
<td>5 mg/kg PO q 12 h</td>
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<td>4 mg/kg PO q 12 h</td>
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<tr>
<td>Azathioprine</td>
<td>Thiopurine analog—disrupts DNA/RNA synthesis</td>
<td>2 mg/kg PO q 24 h × 7–14 days then 2 mg/kg PO EOD</td>
<td>Canine—IMHA[^72]</td>
<td>GI upset, myelosuppression, hepatic necrosis</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Potential drug interactions—allopurinol</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>Alkylating agent—cell-cycle nonspecific</td>
<td>0.02 mg/kg PO q 24 h × 7 d then 0.01 mg/kg 2 mg/cat PO q 48–72 h × 7–14 days then 2 mg/kg PO EOD or 20 mg/m² PO q 14 d</td>
<td>Feline—IMT[^94]</td>
<td>GI upset, myelosuppression</td>
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<td>Feline—I Bd[^92]</td>
<td></td>
</tr>
<tr>
<td>MMF</td>
<td>Purine synthesis inhibitor</td>
<td>10 mg/kg PO q 12 h</td>
<td>Canine—aplastic anemia[^151]</td>
<td>Allergic reaction, diarrhea, weight loss</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 mg/kg PO q 12 h</td>
<td>Feline—IMHA[^153]</td>
<td>Potential drug interactions—FQ, metronidazole, PPIs, GC, cyclosporine</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>Pyrimidine synthesis inhibitor</td>
<td>2–4 mg/kg PO q 24 h</td>
<td>Canine—IMPA[^161]</td>
<td>Lethargy, GI upset, myelosuppression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 mg/cat PO q 24 h</td>
<td>Feline—RA[^165]</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: FQ, fluoroquinolone; GI, gastrointestinal; IBD, inflammatory bowel disease; IMPA, immune mediated polyarthritis; MOA, mechanism of action; PPIs, proton pump inhibitors; PRCA, pure red cell aplasia; RA, rheumatoid arthritis.*
potential for adverse effects remains limited. Ultimately, the goals of any immunosuppressive treatment protocol are to initially achieve disease remission while minimizing adverse effects, followed by a gradual taper of drugs to the lowest doses to maintain disease remission or, in some cases, successful drug withdrawal.

REFERENCES


