Immunosuppressive Therapy for Canine Immune-Mediated Hemolytic Anemia

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Abstract: The mortality for dogs with severe immune-mediated hemolytic anemia (IMHA) is unacceptably high, and better immunosuppressive regimens are needed to increase survival. Understanding the basic immunology of the disease and the mechanisms of action of the available immunosuppressive therapies will help clinicians choose an appropriate immunosuppressive protocol. Prospective, randomized clinical studies must be conducted to evaluate the efficacy and safety of different combined immunosuppressive modalities to treat canine IMHA and improve patients’ outcomes.

At a Glance

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The pathogenesis of canine immune-mediated hemolytic anemia (IMHA) involves the production of immunoglobulin (Ig) that recognizes either a self (autoimmune) antigen or a foreign (immune-mediated) antigen associated with red blood cells (RBCs). This results in sensitization or opsonization of the RBCs and their subsequent destruction by the complement system, the mononuclear phagocyte system, or both.1–3 The interaction of RBC surface-bound Ig with protein crystallizable fragment receptors (FcRs) on different cells in the immune system leads to a wide range of immune responses, including antibody-dependent cellular cytotoxicity, phagocytosis, complement activation, mast cell degranulation, lymphocyte proliferation, antibody secretion, and enhancement of antigen presentation. These responses also promote phagocytosis and clearance of opsonized RBCs by the mononuclear phagocyte system cells and lead to intravascular hemolysis by full activation of complement.1,2,4–5 In severe cases, clinical signs of anemia progress rapidly, and animals may present in shock. Most dogs that succumb to IMHA do so within the first 2 weeks after onset (acute phase).3,6–11 A retrospective study of 60 dogs with IMHA showed a mortality of 52%, with a good long-term outcome in dogs that survived the first 2 weeks of the disease.11

Treatment Options

Current immunosuppressive therapies for IMHA act by a variety of mechanisms, but ultimately they all suppress antibody production by lymphocytes and/or inhibit the clearance of opsonized RBCs by macrophages or lysis by the complement system.5,12–14 Because the half-life of IgG (the antibody class involved in most cases of canine IMHA) in dogs is approximately 1 week, therapies directed only at suppression of antibody production are unlikely to affect the outcome in the acute phase of the disease.13,14 Over the past 25 years, great advances have been made in the field of immunology, especially in the development of new immunosuppressive agents (e.g., cyclosporine, leflunomide, mycophenolate mofetil) that are more potent, more selective, and less toxic than glucocorticoids.5,14,15 The wide array of new drugs offers the opportunity to use combinations that block different pathways of immune activation while selecting agents with non-overlapping toxicity profiles. Nonetheless, IMHA mortality remains high, ranging from 22% to 80%.2,3,6–11,16–18 Moreover, multiple retrospective studies have shown that the addition of cyclosporine, azathioprine, or cyclophosphamide to glucocorticoids...
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The mortality for IMHA is high, with most deaths occurring within the first 2 weeks of clinical disease.

did not improve mortality.6,11

IMHA and its therapy can be divided into three phases: induction of remission, maintenance of remission and prevention of relapse, and management of relapse. The causes of death in dogs that die during the acute phase of IMHA are not well documented, but in addition to lack of response to immunosuppressive agents, they include thromboembolic disease, sepsis, and other side effects of therapy, as well as reactions to antithrombotic therapy and blood transfusions.6,9,18 One way to improve outcomes in the acute phase of IMHA is to focus the induction of immunosuppression on manipulating FcR/Ig interactions, reducing phagocytosis, and inhibiting complement. The causes of death during the maintenance phase of IMHA therapy are also unclear, but they may be related to disease recurrences or side effects of immunosuppression. To improve outcomes and minimize relapses in this phase, maintenance immunosuppressive therapy should be potent, specific, and associated with few or no side effects.

Glucocorticoids

Glucocorticoids remain the mainstay of immunosuppressive therapy for canine IMHA. Their activity is largely derived from their ability to repress the transcription of many genes responsible for encoding proinflammatory cytokines and adhesion molecules that influence immune cell trafficking and cellular interactions. The molecular basis of glucocorticoid action lies in the capacity to diffuse through the cell membrane and bind to cytosolic steroid receptors, which subsequently undergo nuclear translocation and modulate transcriptional activation.5,12,19,20 The main effect of glucocorticoids in the treatment of IMHA is to suppress complement and phagocytosis of opsonized RBCs by interfering with the expression and function of macrophage FcRs, which is an immediate effect.5,20

Prednisone (2 mg/kg bid) is commonly used to induce remission in IMHA, and a variety of dosages and protocols have been advocated.1–3,5–11,16–18 There have been no studies to evaluate the effect of different prednisone dosages on short- or long-term outcomes. Factors that may influence dosing include patient size, body condition, breed, disease severity, concurrent illnesses, and the presence of gastrointestinal (GI) signs at presentation. For induction, the recommended dose is 2 mg/kg bid for dogs weighing less than 6 kg and 1.1 mg/kg or 30 mg/m² bid for those weighing more than 30 kg.2,17 Remission is signaled by a stable or rising packed cell volume (PCV) with no clinical signs of decreased oxygen-carrying capacity over a period of 7 to 14 days. At this time, the dose may be decreased by 25% to 50% (depending on the severity of side effects) and then by 25% every 2 weeks thereafter.2

Once a dose of 0.5 mg/kg sid is reached, the patient can be switched to alternate-day prednisone therapy. As the side effects of prednisone at this point are typically mild, decreasing the dose by 25% every 4 to 6 weeks rather than every 2 weeks is generally advisable. Prednisone administration can be completely discontinued if there are no signs of relapse while the patient is receiving 0.25 mg/kg every other day for 4 to 6 weeks (TABLE 1). A complete blood count (CBC) should be obtained before any decrease in the dose of immunosuppressive agents. A less aggressive tapering protocol may be advised when glucocorticoids are used as the sole immunosuppressive agent. Although most dogs can be completely weaned from glucocorticoids, a few patients may require lifelong, low-dose therapy to maintain remission.2,11 This prednisone protocol is based solely on my clinical experience, and there are no published studies establishing its superiority.

Adverse effects of glucocorticoids include iatrogenic hyperadrenocorticism, GI ulceration and perforation, recurrent infections, sepsis, and thromboembolic disease.21–25 Glucocorticoids may also predispose patients to pancreatitis, although this has not been proven.24 Based on clinical experience, large- and giant-breed dogs are especially sensitive to adverse effects of glucocorticoids. In contrast, most dogs weighing less than 6 kg generally experience only mild adverse effects. Dexamethasone is considered by some clinicians to be superior to prednisone in inducing IMHA remission, but there are no studies to support this opinion, and (anecdotally) GI ulceration and pancreatitis are more common with dexamethasone than with prednisone.13,14 In addition, due to its longer duration of effect in suppressing the hypothalamic–pituitary–adrenal axis, dexamethasone is not appropri-
**TABLE 1  Suggested Immunosuppressive Protocol**

This protocol is for a 25-kg dog with a body condition score of 5/9, using prednisone for induction of remission and azathioprine to help with maintenance, and is based on clinical experience.

<table>
<thead>
<tr>
<th>Day</th>
<th>Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td>Prednisone at 30 mg PO bid or, if the dog cannot tolerate oral medication, dexamethasone at 9 mg IV sid</td>
</tr>
<tr>
<td><strong>Day 5</strong></td>
<td>No GI disturbance for at least 48 hr Maintain prednisone at 30 mg bid and add azathioprine at 50 mg PO sid</td>
</tr>
<tr>
<td><strong>Day 7</strong></td>
<td>PCV is stable or rising for the previous 48 hr and there are no signs of GI disturbance Discharge the patient from the hospital and continue the same medications</td>
</tr>
<tr>
<td><strong>Day 14</strong></td>
<td>PCV is rising but subnormal, with no signs of myelotoxicity/hepatotoxicity or serious glucocorticoid side effects Obtain a CBC and chemistry profile Maintain prednisone at 30 mg bid Decrease azathioprine to 50 mg q48h</td>
</tr>
<tr>
<td><strong>Day 21</strong></td>
<td>PCV is normal, with no signs of myelotoxicity or serious glucocorticoid side effects Obtain a CBC Decrease prednisone to 20 mg bid Maintain azathioprine at 50 mg q48h</td>
</tr>
<tr>
<td><strong>Day 28 (week 4)</strong></td>
<td>PCV is normal, with no signs of myelotoxicity or serious glucocorticoid side effects Obtain a CBC Maintain prednisone at 20 mg bid Maintain azathioprine at 50 mg q48h</td>
</tr>
<tr>
<td><strong>Week 5</strong></td>
<td>No signs of relapse, myelotoxicity, or hepatotoxicity Obtain a CBC and chemistry profile Decrease prednisone to 15 mg bid Maintain azathioprine at 50 mg q48h</td>
</tr>
<tr>
<td><strong>Week 7</strong></td>
<td>No signs of relapse or myelotoxicity Obtain a CBC Decrease prednisone to 10 mg bid Maintain azathioprine at 50 mg q48h</td>
</tr>
<tr>
<td><strong>Week 9</strong></td>
<td>No signs of relapse or myelotoxicity Obtain a CBC Decrease prednisone to 15 mg sid Maintain azathioprine at 50 mg q48h</td>
</tr>
<tr>
<td><strong>Week 11</strong></td>
<td>No signs of relapse or myelotoxicity/hepatotoxicity Obtain a CBC and chemistry profile. Decrease prednisone to 10 mg sid Maintain azathioprine at 50 mg q48h</td>
</tr>
<tr>
<td><strong>Week 13</strong></td>
<td>No signs of relapse or myelotoxicity Obtain a CBC Decrease prednisone to 5 mg q48h Maintain azathioprine at 50 mg q48h</td>
</tr>
<tr>
<td><strong>Week 17</strong></td>
<td>No signs of relapse or myelotoxicity/hepatotoxicity Obtain a CBC and chemistry profile Discontinue prednisone Maintain azathioprine at 50 mg q48h</td>
</tr>
<tr>
<td><strong>Week 23</strong></td>
<td>No signs of relapse or myelotoxicity/hepatotoxicity Obtain a CBC and chemistry profile Decrease azathioprine dose to 50 mg q 3 days</td>
</tr>
<tr>
<td><strong>Week 27</strong></td>
<td>No signs of relapse Obtain a CBC Discontinue azathioprine</td>
</tr>
<tr>
<td><strong>Week 30</strong></td>
<td>No signs of relapse Recommend a CBC q 2 mo for the first year and biannually to triennially thereafter</td>
</tr>
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</table>

*CBC = complete blood count; PCV = packed cell volume.*
ate for alternate-day therapy. Because the main effect of glucocorticoids in treating IMHA is to suppress complement and FcR-mediated clearance of opsonized RBCs, glucocorticoids are the drugs of choice in the induction phase of treatment. However, as glucocorticoids can have severe side effects when used chronically at high doses, they are not ideal for maintaining remission and preventing relapse.

**Human Intravenous Immunoglobulin**

Human IV immunoglobulin (hIVIG) is a sterile, purified IgG preparation manufactured from pooled human plasma that typically contains more than 95% unmodified IgG, which has intact Fc-dependent effector functions, and only trace amounts of IgA and IgM. Several mechanisms of action have been proposed, including antiidiotypic activity, inhibition of autoantibody production, acceleration of autoantibody breakdown and removal, and suppression of complement activation.25–32 Most importantly, hIVIG may reduce Fc-mediated phagocytosis of IgG-coated RBCs.25,26 One study showed that hIVIG binds to canine lymphocytes and monocytes and inhibits RBC phagocytosis.26

hIVIG has been used successfully in multiple studies to treat a small number of dogs with IMHA.31,32 A total infusion of 0.5 to 2 g/kg administered in a divided dose on 2 consecutive days over a period of 6 to 12 hours has been beneficial in some refractory cases of canine nonregenerative IMHA.31 No significant side effects were reported in these limited samples.31,32

Due to its rapid onset of action and potential to block the phagocytosis of sensitized RBCs, hIVIG may be useful in the induction of remission in dogs with severe IMHA. In the few reported cases, the response was often rapid but transient, suggesting that hIVIG is not ideal for maintaining remission.31,32 Indeed, it is potentially immunogenic in dogs, and repeated infusions could induce acute anaphylaxis. There are no studies on the efficacy and the safety of repeated hIVIG infusions in dogs. Although hIVIG is expensive ($600 for a 5-g bottle), it may be practical and economical to use for induction of remission if it significantly decreases hospitalization time and transfusion requirements. Repeated maintenance infusions are currently not recommended.

**Danazol**

Danazol is an androgen derivative used in the early 1990s as an adjunctive treatment for canine immune-mediated thrombocytopenia.33,34 Similar to glucocorticoids, danazol (10 to 15 mg/kg sid) may inhibit binding of the Fc portion of immunoglobulin to FcRs and hence prevent phagocytosis of opsonized RBCs.33,35 However, in one preliminary report of a small, prospective study in dogs with IMHA, adding danazol did not show any significant beneficial effects compared with prednisone alone.35 Danazol is an anabolic steroid and can cause dramatic weight gain in a short time, especially when used in combination with prednisone. Furthermore, danazol is expensive and may be hepatotoxic.36 Due to these side effects of chronic use, danazol is not recommended to maintain remission.

**Splenectomy**

Splenectomy may be considered for patients with IMHA that fail to respond to, or have severe adverse effects from, immunosuppressive therapy or that require long-term, high-dose therapy to remain in remission. The spleen is a major site of autoantibody production and of sequestration and destruction of IgG-sensitized RBCs. A thorough search for an underlying infection is mandatory before proceeding with splenectomy. Preliminary results of a small, prospective, unpublished study showed that splenectomy as an adjunctive therapy is superior to medical therapy alone in reducing mortality and shortening the interval to normal PCVs in dogs with severe IMHA.37 In fact, splenectomy may be effective for both remission induction and maintenance therapy for severe IMHA. It may also be helpful in dogs with multiple acute relapses or in those that only partially respond to medical immunosuppression. However, as the site of clearance of IgM-sensitized RBCs appears to be the hepatic Kupffer cells, splenectomy is unlikely to be beneficial in dogs with positive Coombs’ test results or IgM autoantibodies.38

No large-scale studies have evaluated the short- and long-term safety of splenectomy in dogs. Due to the cost of surgery and risk of complications, splenectomy is rarely recommended as a first-line therapy for severe IMHA. On the other hand, given the encouraging preliminary data, further studies to evaluate the safety and efficacy of splenectomy in the treatment of canine IMHA are warranted.

**QuickNotes**

Glucocorticoids are the mainstay of immunosuppressive therapy for canine IMHA, but they can cause serious side effects.
Plasmapheresis
Plasmapheresis is a process whereby the components in plasma believed to cause or exacerbate disease are removed. The remaining blood components are then combined with replacement plasma or an inert substitute and returned to the patient.\(^\text{39,40}\) The clinical effectiveness of plasmapheresis in the treatment of autoimmune disease may be due to the partial removal of autoantibodies, immune complexes, complement components, proinflammatory agents, and soluble adhesion molecules.\(^\text{39}\) Plasmapheresis is most useful for rapidly reducing plasma concentrations of autoantibodies or immune complexes while other immunosuppressive measures are applied to prolong the effect.\(^\text{39}\) One study reported encouraging results for plasmapheresis in dogs with systemic immune-mediated diseases.\(^\text{40}\) The study suggested that any dog with an acute immune-mediated disease refractory to conventional immunosuppressive therapy can be considered for plasmapheresis.\(^\text{40}\) Although there are no reported clinical trials of plasmapheresis for IMHA, it may be helpful in acute, refractory cases or cases complicated by concurrent systemic infection.\(^\text{41}\) However, availability of plasmapheresis is limited in veterinary medicine, and experience with its use for treating animals with immune-mediated diseases is minimal.

Cyclophosphamide
Cyclophosphamide is a cytotoxic, myelosuppressive alkylating agent. It cross-links DNA helixes to prevent their separation, thus preventing the formation of a DNA template.\(^\text{3,42}\) Cyclophosphamide is toxic to both resting and dividing cells, particularly proliferating immune cells (lymphocytes).\(^\text{5}\) It suppresses both cell-mediated and humoral immunity, and it may suppress mononuclear phagocytic function.\(^\text{5,12}\) Historically and anecdotally, cyclophosphamide has been effective in inducing and maintaining remission in dogs with fulminant IMHA. Several authors have recommended using cyclophosphamide in cases in which intravascular hemolysis or autoagglutination is present, suggesting that it is especially effective in IgM-mediated hemolysis.\(^\text{5}\) However, cyclophosphamide principally targets lymphocyte proliferation and, therefore, is unlikely to have an immediate effect on reducing levels of circulating antierythrocyte antibodies. As such, it is probably ineffective for remission induction during the acute phase of IMHA.

One retrospective study showed no beneficial effect of adding cyclophosphamide to prednisone in 60 dogs with IMHA,\(^\text{11}\) and another study showed higher mortality in dogs so treated compared with dogs that received prednisone alone.\(^\text{\textsuperscript{9}}\) Interpretation of these studies is complicated by their retrospective nature, and it is likely that cyclophosphamide was used only in the more severe cases. However, a small, randomized, prospective trial found no beneficial effects from adding cyclophosphamide to prednisone in the management of acute IMHA and even suggested that cyclophosphamide may adversely affect outcome.\(^\text{\textsuperscript{10}}\) Indeed, several in vitro and in vivo experimental studies suggest that cyclophosphamide may paradoxically augment immune responses through a toxic effect on T regulatory cells.\(^\text{\textsuperscript{5,43}}\) Cyclophosphamide also has serious adverse effects, including gastroenteritis, myelosuppression, sterile hemorrhagic cystitis, and secondary neoplasia.\(^\text{\textsuperscript{5,44,45}}\) Based on this evidence, cyclophosphamide is not recommended for induction of remission in dogs with IMHA, and the risk–benefit profile should be considered carefully before it is used for maintenance therapy.

Azathioprine
Azathioprine is a cytotoxic antimetabolite that is converted to 6-mercaptopurine in the liver. It is a purine analogue that functions as a competitive purine antagonist, thereby inhibiting cellular proliferation.\(^\text{\textsuperscript{5,12,46}}\) Azathioprine is less toxic to resting cells than cyclophosphamide and therefore has fewer side effects. It primarily suppresses lymphocyte activation and proliferation, reducing antibody production. In vivo experimental studies have shown that azathioprine may help to establish antigen-specific tolerance and decrease the risk of relapse.\(^\text{\textsuperscript{47}}\) Azathioprine also suppresses macrophage function, which reduces inflammatory cytokine production and phagocytic efficiency.\(^\text{\textsuperscript{5,5}}\) The onset of action of azathioprine is slow in people but appears to be faster in dogs, with immunosuppressive effects evident in 2 to 4 weeks.

QuickNotes
Due to its rapid onset of action, human intravenous immunoglobulin may be a good choice in the initial management of severe IMHA.
Several studies have suggested a beneficial effect of azathioprine in the treatment of canine IMHA.6,11,17 However, as these studies are retrospective and lack data on azathioprine timing and dosing and because multiple concurrent immunosuppressive and antithrombotic agents were used, it is difficult to attribute the improved survival directly to use of azathioprine. A large, retrospective study showed that in dogs treated for IMHA with the combination of prednisone, azathioprine, and ultralow-dose aspirin (0.5 mg/kg sid), the survival rates at discharge, 1 month, and 1 year were 88%, 82%, and 69%, respectively, which compared favorably with rates in previously reported studies (57%, 58%, 54%).17 Due to azathioprine’s slow onset of action, it is unlikely to play any role in improving short-term survival. The recommended canine loading dose is 2 mg/kg sid for 5 to 7 days, followed by 2 mg/kg every other day for maintenance.17 I generally continue azathioprine maintenance until the patient has been weaned off prednisone for 4 weeks. There is no well-established dose-tapering protocol for azathioprine, but I taper the dose gradually over 2 to 3 months by extending the period of time between doses by 1 day every 4 weeks (TABLE 1).

The most common adverse effects of azathioprine therapy are GI disturbances and myelosuppression, particularly in large dogs.46,48,49 Acute pancreatitis and cholestatic hepatopathy have been anecdotally reported in dogs receiving azathioprine.48,50,51 The prognosis for azathioprine-induced bone marrow toxicity is good if the drug is discontinued before severe myelofibrosis occurs.49 Therefore, a CBC should be obtained every week for the first month of azathioprine therapy and every 4 weeks for the duration of therapy, and the drug should be discontinued immediately if the neutrophil count declines significantly. Due to its slow onset of immunosuppressive action, azathioprine is not a drug of choice for induction of remission. Conversely, due to its selective immunosuppressive effects, few side effects, availability, and relatively low cost, it is a good choice for maintenance therapy.

**Cyclosporine A**

Cyclosporine A (CsA) is a cyclic polypeptide metabolite of the fungus *Tolypocladium inflatum*. It has potent immunosuppressive properties through its blockage of transcription of cytokine genes in activated T cells. CsA inhibits the phosphatase activity of calcineurin, thereby preventing activation of the nuclear factor of activated T cells (NFAT).5,12 CsA also enhances expression of transforming growth factor-β (TGF-β), which is a potent inhibitor of IL-2–stimulated T cell proliferation and generation of antigen-specific cytotoxic lymphocytes.5,52 Studies indicate that CsA blocks the activation of C-Jun N terminal kinases and p38 signaling pathways triggered by antigen recognition, making CsA a highly specific inhibitor of T cell activation.12 In addition, by suppressing the production of interferon γ, CsA may inhibit the phagocytic capabilities of macrophages.5,53

The efficacy of CsA has been demonstrated in cats and dogs undergoing renal transplantation and in dogs with immune-mediated disorders.54 One retrospective study showed improved outcome in dogs with IMHA that received CsA and glucocorticoids compared with glucocorticoids alone, but the difference did not reach statistical significance.8 This finding suggests that CsA may be superior to glucocorticoids alone, as CsA was likely added only in the more severe cases. Preliminary results from a prospective study showed no beneficial effect from combining CsA with prednisone on the survival of dogs with IMHA in the first 28 days after onset.55 However, four of the 19 dogs in the prednisone-only group experienced relapses compared with none in the combination group. This study has not yet been published, and the number of subjects was small. In addition, a relatively small dose of CsA was used (4 mg/kg sid), and there was no mention of monitoring drug levels. Generally, a dosing regimen of 5 to 10 mg/kg sid is recommended for dogs, and plasma concentrations should be periodically monitored to achieve an effective but safe trough level (400 to 500 ng/mL); an optimal level has not been established.5

The most common side effects of CsA in dogs include GI irritation, gingival hyperplasia, antibiotic-responsive dermatitis, and papillomatosis.5,54,56 Malignancies, including
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lymphoma and squamous cell carcinoma, have been noted as well. In addition, anaphylaxis has been reported in people and dogs receiving IV CsA. CsA-associated nephrotoxicosis is a well known complication in people but appears to be rare in dogs and cats whose serum CsA levels are maintained within the therapeutic range (400 to 500 ng/mL). CsA is expensive and requires blood level monitoring, which usually makes it cost-prohibitive for large dogs. However, due to its potent and specific suppressive effects on lymphocyte proliferation and autoantibody production and tolerable side effects even when used chronically, it may be a good choice for maintenance. In addition, due to its suppression of phagocytosis, it may be beneficial during the induction phase of therapy.

Mycophenolate Mofetil
Mycophenolate mofetil (MMF) is a fermentation product of several Penicillium spp that is metabolized completely in the plasma and liver into its active metabolite, mycophenolic acid (MPA). MPA is an effective, reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), which is a key enzyme in de novo purine biosynthesis. Most other cell lines can maintain their function through the salvage pathway of purine biosynthesis alone, but proliferating lymphocytes depend on both the salvage pathway and the de novo pathway. Due to the high specificity of MPA for IMPDH, MPA is a very selective lymphocyte inhibitor. Blockade of IMPDH by MPA was shown to deplete the guanosine pool in lymphocytes and to inhibit T- and B-cell proliferation, differentiation of alloreactive cytotoxic T cells, and antibody responses. Other mechanisms that may contribute to the immunosuppressive effects of MPA include induction of apoptosis of activated T cells and impairment of the maturation of dendritic cells.

MMF has been shown to be safe and effective in prolonging the survival of canine experimental renal allografts and, anecdotally, has been used successfully in several cases of canine immune-mediated disease. The recommended starting MMF dose for dogs is 20 to 40 mg/kg/day PO divided into two or three doses. Experimental studies showed that dogs are especially sensitive to the adverse GI effects (e.g., diarrhea, vomiting) of this drug. There are no reports on the use of MMF to manage canine IMHA, but based on its mechanism of immunosuppressive action, it should be investigated for maintenance of remission.

Leflunomide
Leflunomide is a synthetic isoxazole derivative that is metabolized in the gut and liver. The active metabolite of leflunomide, A77 1726, reversibly inhibits dihydro-orotate dehydrogenase, the rate-limiting enzyme in the de novo synthesis of pyrimidines. Again, lymphocytes need both the salvage and the de novo pathways of pyrimidine synthesis to meet the high demand for pyrimidines during lymphocyte activation and proliferation. Leflunomide specifically suppresses activated T and B cells while other cells maintain their basal cell division. Experimental transplantation studies showed that A77 1726 prevents antibody production. In this study, 26 dogs with different immune-mediated diseases were treated with leflunomide, including six dogs with IMHA. Five of these six dogs responded well to leflunomide therapy, and the one dog that died had severe myelofibrosis that was probably related to the primary disease. The initial dose of leflunomide was 4 mg/kg/day, but the dose was adjusted to obtain a target trough A77 1726 plasma level of 20 µg/mL. Most of the dogs had mild to moderate disease or partial response to conventional therapy, affording sufficient time for leflunomide to exert its immunosuppressive effects.

Leflunomide is not commonly used in veterinary medicine, so adverse effects are not well documented. Adverse effects reported in a small number of dogs were rare and mild, including decreased appetite, lethargy, mild anemia, and self-limiting GI signs. However, most of the dogs in the study were
concurrently receiving other immunosuppressive agents that may have contributed to these effects. Based on this single study of dogs with IMHA, it seems that leflunomide may be an effective immunosuppressive drug with minimal side effects and should be investigated for maintenance of remission of canine IMHA.

Mizoribine
Mizoribine is an imidazole nucleoside, and its active metabolite (mizoribine monophosphate) is a potent inhibitor of IMPDH.\(^5^2,^6^8\) Therefore, like MMF, it blocks purine biosynthesis in B and T cells and inhibits their proliferation. The antiproliferative effect of mizoribine is linked to a decrease in guanine ribonucleotide pools. Mizoribine has been approved in Japan for renal transplant recipients and is currently undergoing clinical testing in Europe as a substitute for azathioprine in human renal transplant recipients. It appears to be safe and (unlike azathioprine) to have minimal myelotoxicity or hepatotoxicity. The availability of this drug is very limited, and little is known about its safety and efficacy in veterinary medicine, but theoretically, it could be employed for the maintenance of IMHA remission.

Management of Relapse
The relapse rate for canine IMHA is not well documented, but retrospective studies suggest that it is relatively high (13% to 20%).\(^8^,^9^,^11^,^1^6,^1^7\) Clinicians must monitor patients for relapse (TABLE 1), make a prompt diagnosis, assess the severity and the acuteness of the relapse, and institute an appropriate immunosuppressive protocol. The choice of regimen depends on the severity of the relapse. A patient with an acute relapse (e.g., drop in PCV from normal to 25% or lower) should be hospitalized for supportive care and aggressively treated to reinduce remission. Immunosuppressive doses of glucocorticoids are the mainstay of therapy for relapse; adjunctive induction therapies for severe, refractory cases include hIVIG, splenectomy, and plasmapheresis.

On the other hand, a patient with a mild relapse while being weaned off immunosuppressive therapy can be managed by increasing the dose to the previous level. This dog can be managed as an outpatient if it is asymptomatic for anemia, but its CBC should be reevaluated in 1 week to assess the efficacy of therapy. Importantly, a drop in PCV for a dog with IMHA does not always signal a relapse. Other causes of a decreased PCV in dogs receiving immunosuppressive therapy for IMHA include sepsis and GI hemorrhage. Failure to identify patients with these complications can be detrimental and potentially fatal.

Conclusion
Canine patients with severe IMHA have a guarded prognosis. Despite the introduction of several new immunosuppressive agents, mortality remains high.\(^2^,^3^,^5^,^7,^1^2–^1^6\) Glucocorticoids are the mainstay of IMHA therapy for induction of remission, but the addition of more potent immunosuppressive agents to prednisone therapy may be warranted in severe cases and in patients with major adverse glucocorticoid effects. Because azathioprine, CsA, leflunomide, and MMF are unlikely to have immediate beneficial effects in dogs with IMHA but may have immediate adverse reactions, I generally wait to add one of these agents to glucocorticoid therapy until the PCV is stable and the dog exhibits no GI disturbances. At my institution, the most common immunosuppressive agents used in combination with prednisone for severe IMHA are CsA and azathioprine. CsA is faster acting and has fewer side effects than azathioprine, but it can be cost prohibitive in large dogs. Preliminary results seem promising for leflunomide and discouraging for cyclophosphamide, but there are few published prospective, randomized trials evaluating the use of various immunosuppressive agents used in combination with prednisone versus prednisone alone.

Immunosuppressive therapy is only one part of a comprehensive approach to improving the outcome of IMHA. Other therapies being studied include antithrombotic agents; one large, retrospective study showed improved survival in dogs with IMHA that were treated with ultralow-dose aspirin to prevent thromboembolic complications.\(^1^7\) The timing, volume, and type of oxygen-carrying support provided by blood products or blood substitutes is another area that requires further investigation.
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1. Which statement regarding dogs with IMHA is correct?
   a. Most dogs die during the maintenance phase of disease management.
   b. All dogs can be successfully weaned off immunosuppressive therapy.
   c. Dogs that survive the first 2 weeks of the disease generally have a good long-term prognosis.
   d. To avoid relapses, dogs should never be weaned off immunosuppressive medications.

2. Which statement regarding glucocorticoid therapy for treating IMHA is correct?
   a. Prednisone at 2 mg/kg bid is a good dose for large- and giant-breed dogs, whereas small-breed dogs should not receive more than 1.2 mg/kg bid.
   b. It has been proven that dexamethasone is superior to prednisone for inducing remission.
   c. Dexamethasone is not appropriate for alternate-day therapy.
   d. Prednisone at doses higher than 2 mg/kg bid is associated with better outcomes.

3. Which statement regarding immunosuppressive agents used to treat canine IMHA is correct?
   a. Azathioprine is a good drug to induce remission, but it can cause bone marrow suppression.
   b. Hepatotoxicity is a common side effect of cyclophosphamide.
   c. Secondary malignancies have been reported in dogs receiving cyclosporine.
   d. Cyclosporine is a potent cytotoxic immunosuppressive drug.

4. Which statement regarding the mechanism of action of immunosuppressive agents is incorrect?
   a. Azathioprine is a competitive purine antagonist.
   b. Mofetil blocks the purine biosynthetic pathway.
   c. Leflunomide blocks the biosynthesis of pyrimidine.
   d. Cyclosporine works by blocking the biosynthesis of pyrimidine.

5. Which is not a reported side effect of cyclosporine therapy in dogs?
   a. GI disturbances
   b. Infection
   c. Development of secondary neoplasia
   d. Aplastic anemia

6. Which is not a side effect of azathioprine therapy in dogs?
   a. Pancreatitis
   b. Bone marrow toxicity
   c. Gingival hyperplasia
   d. GI disturbances

7. Which is not a side effect of glucocorticoids?
   a. GI ulceration and perforation
   b. Increased risk of infection
   c. Bone marrow suppression
   d. Increased risk of thromboembolic disease

8. Which patient is the most suitable candidate for hIVIG treatment?
   a. A dog with a 3-day history of mild weakness, a PCV of 26%, total bilirubin of 1.5 mg/dL, and 2+ spherocytes
   b. A dog experiencing an IMHA relapse with PCV of 24% and a history of receiving an hIVIG dose 1 month earlier
   c. A dog that has been hospitalized for IMHA for 3 days and received prednisone 2 mg/kg bid and three blood transfusions
   d. A dog presenting with acute IMHA, a PCV of 20%, and a history of congestive heart failure

9. Which immunosuppressive therapy is unlikely to be effective in the first 2 weeks of treating IMHA?
   a. Glucocorticoids
   b. Azathioprine
   c. Cyclosporine
   d. Mycophenolate mofetil

10. In dogs with IMHA, administration of which immunosuppressive drug has been associated with a worse outcome in retrospective studies?
    a. Cyclosporine
    b. Leflunomide
    c. Cyclophosphamide
    d. Mycophenolate mofetil

References: