REVIEW

Immunomodulatory drugs and their application to the management of canine immune-mediated disease

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This review summarises the current understanding of immune response and T cell subsets in the context of development of autoimmunity in the dog. Mode of action and rational usage in immune-mediated disease in the dog are discussed for the following drugs: glucocorticoids, azathioprine, cyclophosphamide, ciclosporin, tacrolimus, human intravenous immunoglobulin, vincristine, danazol, leflunomide, mycophenolate mofetil and liposome-encapsulated clodronate. Disease mechanisms are discussed and published evidence for drug efficacy is scrutinised for five important immune-mediated diseases: immune-mediated haemolytic anaemia, immune-mediated thrombocytopenia, myasthenia gravis, glomerulonephritis and inflammatory bowel disease. Future strategies for more refined manipulation of adverse immune responses are presented.

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INTRODUCTION

Immune-mediated diseases (specifically autoimmune diseases) are of major clinical significance in small animal medicine, and the management of these disorders still fundamentally involves medical suppression of the aberrant immune response. The drugs and the immunosuppressive protocols by which they are used in small animals have invariably been adopted from human medicine and applied empirically without a firm evidence base that provides information on the basic pharmacokinetics and immune effects of these agents in cats and dogs. These drugs are almost all unlicensed for use in companion animals but remain widely used despite the well-documented side effects in the recipients and the potential toxicity of some agents for those who administer them. This review examines medical immunosuppression with focus on diseases of the dog. An overview of the immune system and pathogenesis of autoimmunity precedes discussion of the major immunomodulatory drugs and their application to five relatively common canine immune-mediated diseases.

THE IMMUNE SYSTEM

An understanding of the mode of action and rational clinical application of immunomodulatory drugs requires good working knowledge of the body system that the agents are intended to manipulate. Immunology is a rapidly evolving science and our increasing depth of understanding has helped to explain the action of old drugs and to develop new therapies based on inhibition of recently discovered cellular and molecular pathways. In the past decade, there have been two key areas in which there has been rapid advance in understanding (1) the interaction between the innate and adaptive immune system and (2) the key role of regulatory T cell subsets in control of the adaptive immune response.

Innate and adaptive immune system interaction

The innate immune system has traditionally been thought of as a relatively primitive and simplistic means of immune defence, which relies on the non-specific and relatively slow action of epithelial barriers, secreted antimicrobial substances and the phagocytic and natural killer (NK) cells. The key cell of the innate immune system is the dendritic antigen-presenting cell (APC) that undertakes the initial encounter with foreign antigen (e.g. pathogens and allergens) as these substances penetrate mucocutaneous barriers. It is now known that the pathogen–dendritic cell interaction is relatively specific and mediated by various component molecules of the organism (pathogen-associated molecular patterns), which are recognised by APC receptors [pattern-recognition receptors (PRRs) such as toll-like receptors]. The engagement of PRRs initiates specific intracellular signalling

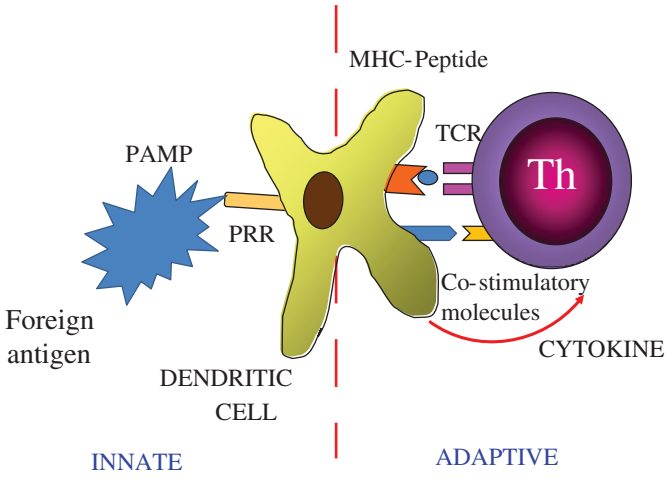

FIG 1. The interface between the innate and adaptive immune system. A foreign antigen (e.g. pathogen) expressing pathogen-associated molecular patterns (PAMPs) is recognised by pattern recognition receptors (PRRs) on the surface of the dendritic antigen presenting cell. This interaction may occur at the tissue site of antigen entry. The antigen is internalised, processed and presented as a small peptide fragment in association with a class II molecule of the major histocompatibility complex (MHC). The dendritic cell travels to the regional organised lymphoid tissue where it encounters an antigen-specific T helper cell (Th) of the adaptive immune system. The T cell is activated by the combination of recognition of MHC peptide by the T cell receptor (TCR), the interaction of costimulatory molecules on the surface of the two cells and the release of stimulatory cytokine by the dendritic cell. The nature of the pathogen and the type of PRRs engaged in turn determines the type of signalling delivered to the T cell and the eventual nature of the adaptive immune response

pathways within the APC that leads to activation of specific genes (Medzhitov 2009). The protein products of these genes in turn are integral to the activation of the T cell by the APC, either by acting as coreceptors in the recognition of presented antigen by the T cell receptor or by providing additional signals to the antigen-specific T cell in the form of soluble messenger proteins (cytokines) that are released from the APC to bind to cytokine receptors expressed by the T cell (Fig 1). In this manner, the innate immune system actually determines the way in which the adaptive immune response to antigen is fashioned (see below). It is very likely that some of the immunomodulatory drugs discussed in this review have effects on these key interactions in the immune response.

T cell subsets

The second major area of immunological development has been the realisation that regulation of the adaptive immune response is undertaken by numerous different T cell subsets. The recognition that T lymphocytes include $CD4^+$ T helper (Th) cells, $CD8^+$ T cytotoxic (Tc) cells and T "suppressor" cells dates from several decades ago. The 1986 description of functional subsets of $CD4^+$ T cells in the mouse has led to an explosion of knowledge in this area, which has fundamentally reshaped our understanding of the immune system and has affected the diagnosis and management of immune-mediated disease and the development of novel therapies and vaccines (Mossman and others 1986). Of greatest importance was the initial recognition of Th1 and Th2 subsets that developed from a common Th0 precursor cell dependent on the signals delivered to that precursor by the APC (as described above).

Th1 cells are characterised by production of the "signature cytokine" interferon (IFN)- γ and their effect is in inducing cell-mediated immunity (CMI) involving processes such as target cell cytotoxicity (e.g. of tumour cells or virally infected cells by Tc) or macrophage killing of intracellular pathogens (e.g. *Leishmania* and *Mycobacterium*). In contrast, Th2 cells produce a panel of cytokines including interleukin (IL)-4, IL-5, IL-9 and IL-13, which permit them to activate B cells allowing transformation to plasma cells and secretion of antibody [e.g. immunoglobulin (Ig) G, IgA or IgE] in the humoral immune response. Other "effector cell" populations have more recently been characterised in experimental rodents and man, including Th17 cells (producing IL-17) that play a role in the immune response to infectious agents (Bettelli and others 2007) and Th9 cells (producing IL-9) that may be important in the allergic immune response (Soroosh and Taylor 2009). There is mounting evidence that dogs and cats also harbour these T cell subsets and particular immune responses and immune-mediated diseases in these species are characterised by cytokine profiles consistent with either Th1/Th17 or Th2/Th9 immunity (Horiuchi and others 2007, Saridomichelakis 2009).

Although the above Th subsets all promote different arms of the immune response, there are other $CD4^+$ T cell subsets that inhibit or suppress immune responses. These are now termed "regulatory" T cells (Treg) but are in effect the same populations as the originally described T "suppressor" cells. A number of different types of Treg are now described including Th3 cells (that produce the cytokine-transforming growth factor β) and induced Treg that are stimulated in the context of specific immune responses. Most important of all are the "natural" Treg that are continuously present within the immune system and have the effect of preventing the activation of potentially damaging

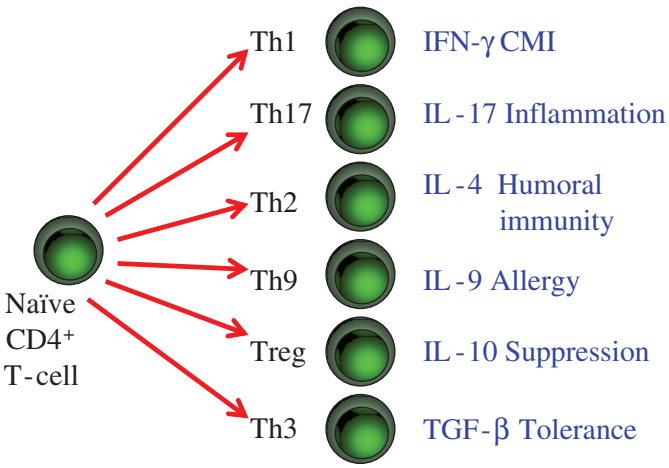

FIG 2. Functional subsets of $CD4^+$ T cells. The functional subsets of T cell expressing the CD4 molecule are defined by the key cytokine that each produces following stimulation by antigen as depicted in Fig 1. The diagram depicts the major subsets, their signature cytokine and their dominant effector role in the adaptive immune response. Some subsets are stimulatory of protective humoral or cell-mediated immunity (CMI), while others have a suppressive or regulatory effect

autoreactive or allergen-reactive T cells (O'Garra and Vieira 2004). These cells are characterised by production of the key immunoregulatory cytokine IL-10 and by expression of a range of other markers (species dependent) including the IL-2 receptor (CD25) and Foxp3. Treg cells have now been identified in both dogs and cats (Biller and others 2007). In man and dogs, it is now clear that one part of the immunology of allergic and autoimmune disease is under-function of these regulatory populations. The functional subsets of $CD4^+$ T cells are summarised in Fig 2.

AUTOIMMUNITY

The drugs discussed in this review are primarily used in the management of canine autoimmune diseases so their rational application also requires an understanding of the nature of autoimmunity. It is clear that autoimmunity is a multi-factorial event involving complex immunological changes. Fundamentally, the latter may involve a lack of the Treg effect in parallel with an inappropriate presentation of autoantigen by APC that permits excessive activation of Th1 cells (for autoimmune diseases involving cytotoxic destruction of target tissue; e.g. canine lymphocytic thyroiditis) or Th2 cells [for autoimmune diseases involving autoantibody production; e.g. canine autoimmune haemolytic anaemia (AIHA) or thrombocytopenia].

These immunological events do not simply occur of their own accord but are precipitated by a complex of underlying predisposing and trigger factors. The most significant predisposing factor is genetic background and it is now recognised that both human and canine autoimmune diseases are in-part genetically determined. Recent investigations have shown clear association between the inheritance of particular alleles of genes of the major histocompatibility complex (MHC) and canine autoimmune diseases such as AIHA and lymphocytic thyroiditis (Kennedy and others 2006a,b). Ongoing work has now begun to search the entire canine genome for susceptibility alleles by genome-wide association studies (Wilbe and others 2010). Other predisposing factors may include age, gender (hormonal background – although this is less clear in dogs than in man) and lifestyle.

The most significant triggering factors are now believed to be infectious agents (Ercolini and Miller 2009). It is increasingly recognised that the immune events leading to clinical autoimmunity may be initiated by preceding infection. In dogs, the best examples of infectious agents known to trigger autoantibody production and autoimmune events are the arthropod-transmitted organisms (e.g. *Leishmania*, *Babesia*, *Ehrlichia*, *Rickettsia* and *Anaplasma*). Other known trigger factors include the administration of some drugs and vaccines.

IMMUNOSUPPRESSIVE DRUGS

This section reviews selected aspects of commercially available immunosuppressive drugs, both those that are in common usage and those that are currently being evaluated for potential application to the treatment of canine disease. Greater detail on the role of these drugs in the management of selected specific disease conditions is given below. Before prescribing any immunosuppressant, the clinician should consider the following questions:

- Is this primary or secondary autoimmunity?
- What underlying non-immunological conditions are present?
- Is immunosuppressive therapy generally effective for this disease? (e.g. see below).
- What adjunctive therapy is required to maximise chances of success and minimise complications (e.g. antimicrobials and antithrombotics).
- "Pharmacogenomics" – are there any breed-specific considerations for use of this drug?
- Are drug interactions likely and does the toxicity profile overlap with existing medications?
- How are efficacy and drug toxicity going to be monitored?
- Is a licensed veterinary product available?
- Is an appropriate formulation/tablet size available?
- Have clear instructions been given to the person administering the medication on safe handling and disposal of wastes?
- Will the owner be able to safely administer the drug at home?

Glucocorticoids

Glucocorticoids act primarily by binding to a cytosolic glucocorticoid receptor (GR), which then translocates to the nucleus, binding specific DNA sequences (glucocorticoid responsive elements) where they act to enhance or inhibit transcription of corresponding genes (Ashwell and others 2000). Recent evidence suggests that more rapid effects are mediated through GRs influencing intracellular signalling, non-specific interactions with cell membranes and specific interactions with membrane-bound receptors (Buttgereit and Schefford 2002). Anti-inflammatory effects of glucocorticoids relate to the stabilisation of cell membranes of granulocytes, mast cells and monocytes-macrophages and inhibition of phospholipase A2 (thereby preventing release of the arachidonic acid metabolites of the cyclooygenase and lipoxygenase pathways). In addition, glucocorticoids prevent release of the pro-inflammatory cytokines IL-1 and IL-6. Effects on complement and rapid downregulation of Fc receptor expression on macrophages [reducing phagocytosis of opsonised red blood cells (RBCs) and platelets] could explain much of the reported early efficacy of glucocorticoids in treating immune-mediated haemolytic anaemia (IMHA, Fig 3) and immune-mediated thrombocytopenia (IMTP; Day and Mackin 2008, Al-Ghazlat 2009).

Immunosuppressive effects in dogs are less clear from published studies but may include reduction in antigen processing and presentation by effects on macrophages and dendritic cells, direct suppression of T cell function (including T cell help for B cells) and reduced affinity of antibody to cell membrane epitopes (Day 2008a). The widespread distribution of GRs and the vast number of genes affected by GR ligation goes some way to explain the severity and wide range of side effects caused by systemic administration of glucocorticoids (Galon and others 2002). The regulation of genes in different directions in naïve versus activated immune cells could also explain the apparently

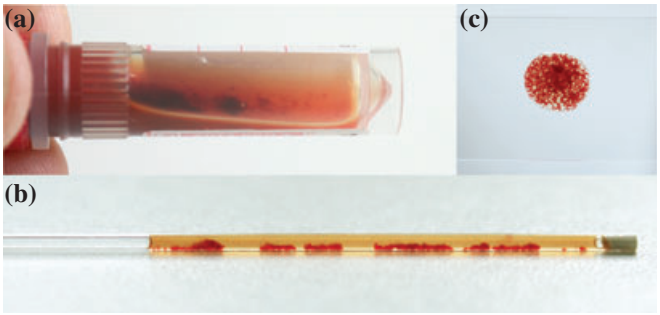

FIG 3 Massive gross autoagglutination of erythrocytes from a dog suffering from IMHA detected in (a) a fresh sample of whole blood in a potassium EDTA tube, (b) the same sample in microhaematocrit tube (icterus is also visible) and (c) the same sample on a microscope slide with addition of a drop of saline

conflicting actions of glucocorticoids on molecules promoting and inhibiting innate and adaptive immunity in experimental models (Galon and others 2002). Veterinary clinicians treating severe canine inflammatory and immune-mediated disease with glucocorticoids often fail in the attempt to achieve disease remission without unacceptable side effects. Clearly, methods of refining glucocorticoid therapy are badly needed. Areas that merit further study and development are use of locally administered steroids, steroid resistance and prophylaxis for potential thrombotic complications.

Currently, prednisolone (prednisone), methylprednisolone and dexamethasone are the most widely used glucocorticoids for systemic effect. Prednisolone has an anti-inflammatory potency roughly equal to hydrocortisone, whereas methylprednisolone and dexamethasone are approximately 5 and 30 times more potent, respectively. Prednisolone and methylprednisolone have an intermediate duration of action and dexamethasone has a long duration of action relative to hydrocortisone. Doses of prednisolone up to 0·5 mg/kg twice daily are considered anti-inflammatory and higher doses immunosuppressive. In reality, there is likely to be a continuum of effect due to the large number of cellular processes influenced by glucocorticoids and such a division is somewhat arbitrary. Immunosuppressive protocols invariably involve tapering the glucocorticoid dose down to zero (according to clinical effect) over several weeks or months. Glucocorticoids should not be abruptly discontinued due to time required for resolution of the iatrogenic suppression of the hypothalamic-pituitary-adrenal axis (HPAA).

Locally administered glucocorticoids In recent years, locally delivered glucocorticoids (e.g. budesonide, beclomethasone and fluticasone) have been developed in an attempt to maintain high affinity for local GRs while reducing systemic side effects. These drugs are particularly suitable for treating local inflammation at mucosal surfaces. Product absorbed into the blood undergoes essentially complete metabolism by cytochrome P-450-dependent mechanisms on the first pass through the liver (Brattsand 1990, Baptist and Reddy 2009). Fluticasone administered by inhalation shows excellent tolerability in treatment of inflammatory airway disease in dogs (Bexfield and others 2006). Clinical signs of secondary hyperadrenocorticism are not described and although there is some suppression of the HPAA, this is much less marked than that occurring with orally administered prednisolone (Cohn and others 2008). Budesonide has been used for treatment of canine inflammatory bowel disease (IBD) and does cause significant HPAA suppression. Although this does not necessarily correlate with signs of glucocorticoid excess (Tumulty and others 2004) such symptoms have been reported in some dogs by other authors (Battersby and others 2007). Prospective controlled studies on locally administered steroids in dogs are required.

Identifying glucocorticoid-resistant individuals In man, glucocorticoid resistance is a major problem, with treatment failure occurring in up to 30% of patients treated for inflammatory disease (Creed and others 2009). Lymphocyte sensitivity to glucocorticoids varies widely between individuals. Glucocorticoid resistance-related treatment failure in human patients with ulcerative colitis may be predicted by measuring the sensitivity of their T lymphocytes to glucocorticoids in vitro (Hearing and others 1999). IL-2 receptor blockade using a monoclonal antibody can induce disease remission (allowing a significant proportion of patients to avoid colectomy) by effectively resensitising the lymphocytes to glucocorticoids in these patients (Creed and others 2003, 2006). A small pilot study of lymphocytes obtained from seven healthy dogs identified one glucocorticoid-resistant individual within the group (Oliver Garden, personal communication), but the true incidence of glucocorticoid resistance in dogs is unknown. Allenspach and others (2006a,b) have suggested that a significant proportion of dogs with IBD may be glucocorticoid resistant and that mucosal expression of the drug efflux pump P-glycoprotein may help identify these individuals.

Thrombosis prophylaxis An excess of endogenous glucocorticoid increases the incidence of thrombotic disease. This is evidenced by the association between spontaneous hyperadrenocorticism and thromboembolism (Nichols 1994). There are important abnormalities in haemostatic parameters in both dogs and people with hyperadrenocorticism, but the role of these abnormalities in the pathogenesis of thromboembolism is far from clear (Chastain and Panciera 2002, Erem and others 2009). Interpretation of a recent small study to investigate hypercoagulability in canine hyperadrenocorticism using thromboelastography was hampered by the nature of the control population (Rose and others 2009). In contrast, administration of prednisone to healthy beagles clearly induced a hypercoagulable state (again assessed by thromboelastography; Rose and others 2008) and chronic administration of glucocorticoids was shown to significantly impair fibrinolysis in human renal and cardiac transplant recipients (Patrassi and others 1997, Sartori and others 2000). It is therefore appropriate to advise clients of the potential for increased thromboembolic risk when prescribing glucocorticoids to dogs, especially where there are pre-existing risk factors for thromboembolism (such as IMHA).

Glucocorticoids as "standard of care" Glucocorticoids are very widely used in veterinary medicine, forming a universally accepted cornerstone of therapy for many conditions.

Although two recent evidence-based reviews concluded that there is a firm basis for the use of oral glucocorticoids in management of canine atopic dermatitis, the reviewers conceded that most of the evidence derived from trials in which a glucocorticoid treatment group was used as the active control arm in randomised controlled trials testing the efficacy of other drugs (Olivry and Mueller 2003, Olivry and others 2010). To the best of our knowledge, glucocorticoids have never been subjected to rigorous evaluation by randomised double-blinded placebo-controlled trials for treating canine immune-mediated disease. For potentially life-threatening conditions, withholding glucocorticoids to compare the effect of another immunosuppressive drug against placebo is cited as unethical (Rozanski and others 2002). Some might consider the evidence of glucocorticoid efficacy from observational data and numerous retrospective studies sufficiently compelling as to make the actual need for prospective placebo-controlled evaluation of glucocorticoids as ludicrous as the satirical suggestion that there is a need to prospectively evaluate the efficacy of a parachute for preventing death when jumping from a plane (Smith and Pell 2003)! Yet, glucocorticoid therapy itself carries considerable morbidity, and treatment failure due to client perception that glucocorticoid side effects are as bad as the disease being treated is not uncommon. There is no easy way around this situation but we urge careful attention to the control groups used when interpreting data from such clinical trials.

Azathioprine

Azathioprine (Imuran, Glaxo Smith Kline) is a cytotoxic synthetic imidazole derivative of 6-mercaptopurine (6MP). Both are thiopurines, which interfere with purine synthesis and result in production of fraudulent nucleotides. Hence, DNA and RNA syntheses are inhibited, and mitosis and cellular metabolism is disrupted. Two studies found that azathioprine does not reduce serum immunoglobulin concentrations (Ogilvie and others 1988, Rinkardt and others 1999). Rinkardt and others (1999) also reported no reduction in circulating blood lymphocytes or specific subsets of these cells. However, these observations were only made at 14 days after commencing the therapy, and it is generally accepted that azathioprine has a "lead-in time" of 11 days before having clinical effect. The main effects of this drug are thought to be on CMI with a reduction in lymphocyte number and T cell-dependent antibody synthesis (Beale 1988, Miller 1992, Day 2008b). Azathioprine has long been used in combination with glucocorticoids, with which it is thought to have a synergistic effect, to allow more rapid dose reduction or tapering of glucocorticoids (the "glucocorticoid sparing" effect). Speed of onset of immunosuppression induced by azathioprine alone is reported to be variable. Ogilvie and others (1988) documented a reduction in T cell blastogenic response to mitogens at 7 days, whereas Beale (1988) suggested a lag period of 3 to 5 weeks. Azathioprine has been extensively used in management of canine immune-mediated disease, with relatively low cost and good tolerability making it an attractive choice. A few large retrospective studies lend limited support to its use but there are no prospective controlled studies.

The principle adverse effects of azathioprine are myelosuppression, acute pancreatitis, hepatopathy and gastrointestinal distress (Beale 1988, Houston and Taylor 1991, Rinkardt and Kruth 1996, Kidd and others 2004). Safety and efficacy of azathioprine therapy in dogs might be refined by considering drug metabolism in more depth: azathioprine is initially converted in the liver and other tissues to 6MP. Thiopurine methyltransferase (TPMT) is an important enzyme in the metabolism of 6MP and can be measured in canine erythrocytes. Variation in TPMT activity correlates with clinical outcomes in human patients receiving azathioprine, with high TPMT activity associated with reduced efficacy and low TMTP activity associated with increased risk of bone marrow toxicity. Notably, significant breed-related variation in TMTP activity has been documented in dogs, with TMTP activity being much lower in giant schnauzers and much higher in Alaskan malamutes than in other breeds (Kidd and others 2004). Six single-nucleotide polymorphisms have been found to account for much of the variation in TMTP activity in the canine population (Salavaggione and others 2002).

Cyclophosphamide

Cyclophosphamide (Endoxana; Baxter Oncology) is a cytotoxic alkylating agent that cross-links DNA, preventing its separation. It is toxic to resting and dividing cells, particularly proliferating lymphocytes. It suppresses CMI and humoral immunity (Al-Ghazlat 2009) but paradoxically increases the severity of inflammatory disease and reduces regulatory T cell numbers in some experimental models (Su and others 2006). Cyclophosphamide has been used extensively in the dog for management of primary IMHA and sporadically for other immune-mediated diseases. It has been proposed that the primary indication for cyclophosphamide therapy in the dog should now be for cancer therapy. As for most other immunosuppressive agents, there is a lack of published efficacy data, but studies suggesting increased morbidity and the increased incidence of serious adverse effects of cyclophosphamide (e.g. haemorrhagic cystitis and myelosuppression) do not justify its use as an immunosuppressive agent.

A note of caution regarding prescribing and nomenclature of cytotoxic agents Fatal overdoses of cyclophosphamide and azathioprine have occurred in dogs when these drugs have been dispensed in error in place of ciclosporin and azithromycin, respectively. Terminology coined from an English translation of an article on cyclophosphamide in a Chinese journal seems particularly apt to describe the consequences of such an error, i.e. "haematological damnification" (Yin and others 2005).

Ciclosporin and tacrolimus

Ciclosporin (Atopica™, Novartis; a veterinary medicine licensed for treatment of atopic dermatitis in dogs) exerts its dominant effects by blocking transcription of genes required for T cell activation, notably those encoding a number of immunoregulatory cytokines including IL-2. It does this by binding to the molecule cyclophilin (present in high concentrations in the cytoplasm of T cells) forming a complex that prevents the activation of calcineurin that would normally occur when T cell receptor engagement with antigen triggers an influx of calcium into the cell. In normal

cells, activated calcineurin then acts on nuclear factor of activated T cells, which migrates to the nucleus, directly inducing cytokine gene transcription. Reduced IL-2 production induced by ciclosporin reduces clonal proliferation of the T cell and consequently of B cells. Production of IL-3, IL-4, granulocyte colony-stimulating factor and tumour necrosis factor (TNF)-α is also reduced and there are indirect effects on granulocytes, macrophages, NK cells, eosinophils and mast cells. Ciclosporin also affects granulocytes and platelets via interference with calcium-dependent exocytosis of granule-associated serine esterases (Day 2008b).

Ciclosporin is widely used in transplantation medicine in people, cats and dogs and is being applied to an ever-increasing spectrum of immune-mediated and inflammatory diseases in the dog. Ciclosporin occupies a unique position in the battery of immunosuppressive drugs available to veterinarians as the only such drug licensed for systemic administration to dogs. The license is specifically for treatment of atopic dermatitis, supported by excellent data showing efficacy. Recent reviews and a meta-analysis of 10 prospective clinical trials concluded that ciclosporin was highly effective compared with placebo and was as effective as prednisolone for treatment of atopic dermatitis in the dog (Steffan and others 2006, Olivry and others 2010). A small prospective placebo-controlled study also showed the efficacy of ciclosporin for treatment of anal furunculosis in dogs (Mathews and Sukhiani 1997), and several other uncontrolled studies also support use of this drug for this clinical indication (Griffiths and others 1999, Patricelli and others 2002, Hardie and others 2005). However, despite numerous case reports and case series, prospective controlled studies showing efficacy for other conditions are either lacking or do not support its use except in transplantation medicine, atopic dermatitis and for anal furunculosis.

Ciclosporin is generally well tolerated in dogs but a range of adverse reactions are reported (most commonly gastrointestinal signs) that are either transient or resolve on discontinuation of the drug (Robson 2003). Published reports document serious opportunistic infections (Cook and others 1994) and emergence of neoplasia (Blackwood and others 2004, Callan and others 2005) in dogs receiving ciclosporin. Although ciclosporin is a very potent T cell inhibitor, and reports of parallel experience with the drug in man exist, it is not clear whether the incidence of these problems is greater in dogs receiving ciclosporin than any other immunosuppressant. Based on work in man, Thomason and others (2009) recently presented preliminary data on three dogs, suggesting that ciclosporin may increase platelet procoagulant activity. Ciclosporin is metabolised by cytochrome P450, leading to numerous potential drug interactions, and these are detailed in the product data sheet. Such interaction may be exploited by concurrent use of ketoconazole which increases ciclosporin blood levels, allowing lower doses to be used to reduce costs, particularly in large breed dogs (Mouatt and others 2002). Atopica™ is a microemulsified form of ciclosporin and the data sheet does not carry the same recommendations for monitoring of blood ciclosporin concentrations that applied to previous products.

Ciclosporin ointment (Optimmune; Shering-Plough) is licensed for treatment of keratoconjuntivitis sicca in the dog. Tacrolimus (Protopic; Astellas), a closely related but unlicensed drug, is also available as an ointment. Use of Tacrolimus for canine atopic dermatitis is supported by prospective controlled studies (Olivry and others 2010) and it is also used in canine anal furunculosis (Misseghers and others 2000, Stanley and Hauptman 2009). Tacrolimus inhibits IL-2 and IL-4 production by interference with calcineurin (Day 2008b).

Human intravenous immunoglobulin

Human intravenous immunoglobulin (IVIG; numerous preparations) has broad but incompletely understood immune-modulatory effects in people and has multiple clinical indications supported by controlled studies (Tha-In and others 2008). In dogs, in vitro evidence that IVIG competitively inhibits the binding of canine IgG to monocytes by saturation of Fc receptors provides rationale for use in IMHA and IMTP by prevention of phagocytosis of antibody-coated erythrocytes and platelets, respectively (Reagan and others 1998). These authors also documented IVIG binding to canine T (CD4 and CD8) and B cells in vitro. Human data suggest that IVIG interferes with Fas (CD95) – Fas ligand (CD95R) induced apoptosis in lymphocyte-mediated cytotoxicity of target cells (Viard and others 1998), providing a possible explanation for suggested efficacy in canine immune-mediated skin diseases such as erythema multi-forme (Nuttall and Malham 2004, Rahilly and others 2006, Trotman and others 2006). Additional mechanisms of immunosuppression in people have recently been reported. The Fab2 portion of IgG inhibits T cell proliferation in the absence of costimulation or accessory cells, and physiological amounts of IgG in plasma have significant regulatory effects on the proliferative responses of T cells (MacMillan and others 2009). Anti-inflammatory properties of IVIG have been linked to sialylation of the Fc fragment, guiding development of an IVIG replacement (Kaneko and others 2006, Anthony and others 2008). Better understanding of mechanisms leading to eventual development of synthetic replacements for the active components of IVIG is important given that the increasing number of indications for IVIG usage in human medicine is putting pressure on available supply (Bayry and others 2007). Although IVIG has been used for various conditions in dogs, efficacy data are limited to case reports and two small controlled studies. While this situation persists, use of IVIG may be difficult to justify on ethical (and financial) grounds at times when demand is high for human patients. IVIG has recently been shown to promote hypercoagulability and an inflammatory state in healthy dogs in a small controlled trial (Tsuchiya and others 2009) and this would question the application of this treatment to dogs with IMHA in which a hypercoagulable state is already recognised (see below).

Vincristine

Vincristine is not used for an immunosuppressive effect but has application as an adjunct therapy in the management of IMTP in the dog. Vincristine binds to tubulin and disrupts assembly of the mitotic spindle in mitosis. Used at lower doses than typically employed in cancer chemotherapy, it increases platelet counts by stimulating megakaryoctes and impairs phagocytosis of opsonised platelets by impairing microtubule assembly in

macrophages (Lewis and Meyers 1996). At such doses (0·02 mg/kg IV), vincristine is not normally myelosuppressive. Whether vincristine has an effect on platelet function in dogs with IMTP is unknown. One study demonstrated that in vivo platelet function was not altered by vincristine in clinically healthy dogs (Mackin and others 1995). Another study revealed abnormal in vitro platelet function in dogs with lymphoma treated with vincristine (Grau-Bassas and others 2000).

Danazol

Danazol (Danocrine, Winthrop Pharmaceuticals) is a synthetic androgen which is reported to downregulate macrophage Fc receptor expression, reduce antibody binding to erythrocytes, stabilise erythrocyte membranes and alter T cell homeostasis. It has been used as an adjunctive agent with glucocorticoids in canine IMHA and IMTP, but its efficacy is not supported by published reports and usage is now uncommon (Al-Ghazlat 2009). Danazol can be hepatotoxic in dogs.

Leflunomide

Leflunomide (Arava7, Hoechst) is an isoxazole immunomodulatory drug, licensed in some countries for treatment of human rheumatoid arthritis. The primary metabolite reversibly inhibits dihydro-orotate dehydrogenase, the rate-limiting enzyme in de novo pyrimidine synthesis. At high concentrations, cytokine and growth factor receptor-associated tyrosine kinase activity are inhibited (Gregory and others 1998a, Bianco and others 2009). T and B cell proliferation is inhibited and the drug has significant anti-inflammatory effects. Work in rodent models of contact allergy and inflammatory brain disease also suggests that leflunomide may induce regulatory T cells (Korn and others 2004, Weigmann and others 2006) and that induction of CD86 expression and IL-10 production by microglial cells could play a role in the CNS effects (Korn and others 2004). Leflunomide was first used in dogs as an adjunctive agent in renal transplantation (McChesney and others 1994, Lirtzman and others 1996) but has been applied to treatment of a range of immune-mediated and inflammatory diseases (Gregory and others 1998b, Colopy and others 2010). It is used primarily in cases refractory to conventional medications or where glucocorticoid use is contraindicated (Bianco and Hardy 2009) or glucocorticoid side effects prove unacceptable. Efficacy is also reported in canine reactive histiocytosis (Affolter and Moore 2000). Despite having a long elimination half-life and potential hepatotoxicity and myelotoxiticty, these effects seem rare. Controlled studies performed in the dog are confined to those in renal transplantation, some involving leflunomide analogues (Kyles and others 2001, 2003).

Mycophenolate mofetil

Mycophenolate mofetil (MMF; CellCept; Roche Laboratories) was developed as an alternative to azathioprine with reduced myelotoxicity and hepatotoxicity and is now widely used in multi-drug regimes for prevention of human renal allograft rejection (and more widely in transplantation medicine) and for treating several human immune-mediated diseases (Danovitch 2005, Appel and others 2007, Abelson and others 2009). MMF is metabolised in the plasma and the liver into mycophenolic acid (MPA), a reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), a key enzyme in de novo purine biosynthesis. This pathway is essential for lymphocyte proliferation; hence, T and B cell proliferation, differentiation of Tc cells and antibody responses are inhibited. MPA may also induce apoptosis in activated T cells and impair dendritic cell maturation (Mehling and others 2000). Clinical use in the dog has emerged from extensive use in canine renal and bone marrow transplantation models. Significant potential advantages of MMF as an immunosuppressive agent in the dog include availability of a parenteral preparation, rapid onset of IMPDH inhibition (and hence immunosuppression) occurring 2 to 4 hours after dosing (Langman and others 1996) and low toxicity with signs primarily limited to gastrointestinal effects (Platz and others 1991), although mild suspected allergic reactions have been reported with the parenteral preparation (Dewey and others 2001). Anecdotally, on-line case discussion forums for internal medicine specialists suggest that veterinary clinical usage of MMF for treatment of refractory immune-mediated disease is now quite widespread, especially for acquired myasthenia gravis (AMG; Dewey and others 2010), IMHA and pemphigus vulgaris. There is a need for prospective studies to justify and refine this usage and for preliminary studies to be documented in the literature.

Clodronate

Clodronate is a bisphophonate that has been used in management of hypercalcaemia associated with vitamin D intoxication in dogs (Mellanby and others 2005, Ulutas and others 2006). Liposome-encapsulated clodronate (LC; Clodrosome; Encapsula Nano Sciences) is preferentially phagocytosed by macrophages and dendritic cells which then undergo apoptosis. LC effectively reduces the severity of different autoimmune diseases in murine model systems. A recent study showed that LC induces killing of canine splenic macrophages and dendritic cells in vitro and that infusion of LC inhibits clearance of opsonised RBCs in normal dogs. LC infusion was well tolerated in dogs, and survival rates were improved in a small group of dogs with IMHA (although these patients also received other immunosuppressants) when compared with historical controls (Mathes and other 2006). The authors suggest that LC may have a role in treating antibody-mediated cytopenias where macrophages play an important role by rapidly stopping erythrocyte or platelet destruction while gaining time for slower acting immunosuppressants to become effective. Based on these findings, prospective randomised placebo-controlled trials of LC are in progress at Colorado State University (CSU) for management of IMHA in dogs (Lunn 2009). CSU is also evaluating LC for efficacy in the management of malignant histiocytosis in dogs.

PATHOPHYSIOLOGY AND MANAGEMENT OF CANINE IMMUNE-MEDIATED DISEASES

Choice of treatment regime should be based on knowledge of the pathophysiology of the disease in the dog and any available

published evidence for safety and efficacy of the selected drug for that condition, preferably in a clinical setting. In vitro evidence for drug efficacy does not always translate to clinical response. Where studies are lacking, drugs showing efficacy for other immune-mediated conditions in the dog may be considered. Careful scrutiny of management of analogous diseases in other species may also suggest drugs that might be applied to the canine condition, provided the drug has shown a reasonable safety margin in dogs. Where possible, drugs will only be discussed in this section with reference to published reports, with emphasis placed on prospective placebo-controlled studies if available.

Immune-mediated haemolytic anaemia

Key points Reinstating immune tolerance in IMHA is challenging and mortality rates for this condition remain high, primarily due to thrombotic complications.

Bias in case and drug selection Studies of IMHA and most other immune-mediated diseases are almost invariably performed at referral institutions. This may exclude patients that respond quickly to treatment in general practice, selecting for inclusion of more severely affected patients. Retrospective studies provide useful information regarding clinicopathological variables associated with increased mortality, but their use for identifying optimal therapy is fraught with difficulty due to bias in patient selection for a given drug, lack of standardised immunosuppressive and anticoagulant protocols and variation in the timing of haematological monitoring and follow-up. Awareness of high mortality early in the disease course, client anxiety and institutional culture may prey on the patience of a clinician waiting for a response to monotherapy, resulting in selection of an additional agent. This could lead to errors in interpretation in two ways. More severely affected patients may be selected to receive a second drug, with increased mortality in that group then attributed to the drug rather than the disease. Conversely, apparent response to a drug administered in addition to glucocorticoids may simply reflect a group of patients surviving long enough to receive a second drug.

Pathophysiology IMHA involves antibody-mediated cytotoxicity (a classical Gell and Coombs type II hypersensitivity response) resulting in intravascular lysis of erythrocytes following full activation of the terminal pathway of the complement system or extravascular haemolysis following opsonisation and phagocytosis by macrophages in the liver, spleen or elsewhere. Autoantibodies have been identified in canine IMHA that are specific for erythrocyte membrane glycoproteins (glycophorins), the erythrocyte anion exchange channel (band 3) and spectrin, a component of the cytoskeleton (Day 1999). Such autoantibodies have also been recognised in normal dogs and may be involved in normal removal of senescent erythrocytes (Barker and others 1991, Christian and others 1993). However, in some cases, the antibody on the erythrocyte surface may not be bound to native self-antigen but to self-antigen modified or exposed by hapten (drug or pathogen) or to non-specifically attached foreign antigens. This is secondary IMHA and there are numerous reported associations with other disease conditions. Recent investigations have suggested that the pattern of Coombs test reactivity when performed with monovalent sera may help distinguish primary from secondary IMHA (Warman and others 2008). Concurrent IMTP is reported in some dogs with IMHA (Jackson and Kruth 1985) and a smaller proportion may have concurrent immune-mediated neutropenia (Day and Mackin 2008).

There have been numerous retrospective studies of IMHA evaluating prognostic factors and immunosuppressive treatment regimes. However, the pathophysiology of IMHA is complex, involving both a systemic inflammatory response characterised by increases in acute phase proteins (Tecles and others 2005, Mitchell and others 2009) and a leucocytosis, the magnitude of which correlates with the severity of post-mortem examination pathology in multiple organs (McManus and Craig 2001). It has long been recognised that thromboembolic disease that accompanies or follows treatment for immune-mediated haemolysis is a dominant cause of mortality (Klein and others 1989, Klag and others 1993, McManus and Craig 2001, Scott-Moncrieff and others 2001, Carr and others 2002, Thomson and others 2004). Reasons for the hypercoagulable state are poorly understood but significant cross-talk between the inflammatory and coagulation cascades is likely to be a factor in the acute phase of the disease (Smith 2007). Despite the occurrence of antiendothelial antibodies in human autoimmune disease, they were not detected in 21 dogs with IMHA (Wells and others 2009). A circulating "lupus-type anticoagulant" may have contributed to pulmonary thromboembolism in one dog in which haemolysis was one manifestation of systemic autoimmunity (Stone and others 1994). Parameters for defining hypercoagulability in dogs have not been standardised. In one study, the platelets in IMHA dogs were more activated and hyperresponsive compared with those of control dogs, suggesting that platelets may be more likely to contribute to prothrombotic events (Weiss and Brazell 2006). Hypercoagulability compared with normal control dogs can be documented by thromboelastograpy (TEG). In a recent retrospective study (in which all dogs with IMHA received glucocorticoids and some received antithrombotic therapy), Sinnott and Otto (2009) found that the majority of patients evaluated by TEG were hypercoagulable; however, those classified as normocoagulable by TEG had an increased mortality rate, all being euthanased with suspicion of pulmonary thromboembolism. A prospective controlled study on use of TEG to identify hypercoagulability in dogs with IMHA before treatment with immunosuppressants, blood products or anticoagulants is underway (Fenty and others 2008). Clearly, once the optimal combination of laboratory tests has been defined that will accurately predict thrombotic risk, clinicians will be in a better position to evaluate thromboprophylactic regimes and target them to the highest risk patients. Suffice it to say that this work is of paramount importance for improving survival in IMHA, but a detailed critique of antithrombotic agents is beyond the scope of this review and discussion will be confined to immunosuppressants in management of this disease.

Therapy Drugs that have received prospective clinical evaluation for canine IMHA are high-dose IVIG, ciclosporin, danazol and cyclophosphamide. All these drugs were administered in conjunction with glucocorticoids. Following retrospective studies

suggesting that IVIG is beneficial in some dogs with IMHA (Kellerman and Bruyette 1997) and non-regenerative anaemia (Scott-Montcrieff and others 2001), respectively, a recent blinded randomised prospective study was performed in dogs with IMHA receiving glucocorticoids as the sole immunosuppressive agent in addition to low-molecular weight heparin. The addition of IVIG offered no benefit over placebo in terms of initial response and hospitalisation time (Whelan and others 2009). Ciclosporin was evaluated in a small placebo-controlled study (all 38 dogs received prednisolone and heparin), but there was no increase in survival at 28 days (Husbands and others 2004). A single citation describes recovery of one dog (of eight dogs enrolled in the study) receiving ciclosporin as the sole immunosuppressive agent (Wohl and Moore 1996). In another study (16 dogs), danazol or placebo were administered in combination with prednisolone and azathioprine, but there was no effect on mortality (Miller 1997). Cyclophosphamide has also been evaluated in a small prospective study (18 dogs, not placebo-controlled) and showed no beneficial effect over prednisolone alone in initial management of acute IMHA (Mason and others 2003). Retrospective studies have suggested that cyclophosphamide used as an adjunct agent is associated with an increased relative risk of death (Grundy and Barton 2001) or shortened survival times (Burgess and others 2000) compared with other drugs, but it has been suggested that cyclophosphamide was given to dogs with more severe clinical illness that were therefore at higher risk of death (Reimer and others 1999).

Azathioprine has never been evaluated prospectively for canine IMHA, but several retrospective studies suggest a beneficial response over prednisolone alone (Allyn and Troy 1997, Reimer and others 1999) or over prednisolone and cyclophosphamide (Reimer and others 1999, Burgess and others 2000). One study observed that beneficial effects on short- and long-term survival were only seen when combined prednisolone and azathioprine therapy was used concurrently with ultra-low-dose of 0·5 mg/kg/day aspirin (Weinkle and others 2005). A further retrospective study of 149 dogs treated with prednisolone and azathioprine (Piek and others 2008) prompted unresolved questions over statistical methods (Elwood and Polton 2008). Use of MMF has been reported as a rescue agent in a dog with aplastic anaemia (Yuki and others 2007) and in combination with prednisolone therapy in eight dogs with IMHA. Notably, seven of these dogs achieved clinical remission for at least 4 months (Nielsen and others 2005).

Immune-mediated thrombocytopenia

Key point Some dogs with IMTP show few signs of systemic illness and most respond favourably to immunosuppressive therapy.

Pathophysiology IMTP is caused by autoantibody against platelet surface membrane antigens, in particular, glycoproteins IIb and IIIa. Comparative aspects of canine and human IMTP have been reviewed in some detail by Lewis and Meyers (1996). IgG antibodies are most frequent and antibodies may sometimes be directed against megakaryocytes in addition to circulating platelets. The role of complement in platelet destruction in dogs is assumed but unproven. Flow cytometric assays for platelet surface-bound IgG are sensitive but not specific for a primary autoimmune process, with immunologically mediated thrombocytopenia occurring in many disease states (Lewis and Meyers 1996). Recognising conflicting results regarding the utility of bone marrow aspiration, antiplatelet antibody detection and study of platelet indices in reaching a diagnosis and formulating a prognosis in IMTP (see Lewis and Meyers 1996), several investigators have revisited these issues (Wilkerson and others 2001, Miller and Lunn 2007, Bommer and others 2008, Dircks and others 2009). There was no consensus on the value of bone marrow evaluation. Lower platelet counts and absence of an increase in mean platelet volume were associated with a higher probability of primary IMTP. However, Bommer and others (2008) concluded that measuring platelet volume indices did not aid the interpretation of thrombocytopenia in terms of the underlying disease process. For many clinicians, reaching a diagnosis of primary (as opposed to secondary) IMTP still remains a process of exclusion but is critical in guiding therapy.

Important additional considerations taken from human IMTP in guiding therapy are the dominance of the spleen as the primary site of platelet removal (Lewis and Meyers 1996) and endothelial cell dysfunction (spontaneous leakage). In contradistinction from IMHA, a marked systemic inflammatory response and a tendency to thromboembolic disease are absent in isolated primary IMTP. In fact, some cases with profound thrombocytopenia remain free from life-threatening haemorrhage or any other symptoms and are discovered serendipitously. Careful choice of drugs in such patients is critical to achieve speedy resolution of the problem without owner perception that the side effects of the drugs are worse than the untreated disease.

Therapy Based on retrospective observations, the majority of dogs with IMTP will attain a platelet count of $>50\times10^9$/l within 7 days of commencing immunosuppressive glucocorticoid therapy (Lewis and Meyers 1996). The only drugs evaluated prospectively in a controlled manner for management of IMTP are vincristine and IVIG. Efficacy of vincristine in the management of canine IMTP, either as a sole agent or in combination with glucocorticoids, was first suggested by Greene and others (1982) based on a small case series. Latterly, vincristine has been regarded as adjunctive therapy for stimulating platelet production. In this capacity, vincristine therapy for IMTP has been evaluated in a small prospective non-randomised unmasked study (Rozanski and others 2002). Dogs that received a single dose of vincristine in addition to glucocorticoid therapy had a significantly faster rise in platelet count and shorter hospitalisation times compared with dogs treated with glucocorticoids alone. Dogs that received vincristine 7 days after prednisolone therapy was initiated and had failed to elevate platelet count also appeared to respond. Side effects from vincristine were uncommon and adequate platelet function was suggested by absence of haemorrhage in all dogs once platelet numbers increased to $>40\times10^9$/l.

The use of IVIG was described in five dogs with severe IMTP (Bianco and others 2007). All these patients were receiving prednisolone and received IVIG early in the course of disease. The same authors recently evaluated IVIG in a prospective,

randomised, double-blinded placebo-controlled trial. All dogs received prednisolone, and dogs treated with IVIG had significantly faster recovery in platelet count and shorter hospitalisation times (Bianco and others 2009). Despite reported usage, the efficacy of other agents (cyclophosphamide, azathioprine and danazol) in canine IMTP is not documented by controlled studies or limited to very small numbers of cases (Williams and Maggio-Price 1984, Jackson and Kruth 1985, Bloom and others 1989, Jans and others 1990, Roseler and Mason 1994). Leflunomide monotherapy eventually maintained normal platelet counts in two of three dogs previously receiving other therapies (Gregory and others 1998b) and a combination of leflunomide and IVIG was recently described for successful management of Evans' syndrome in a dog (Bianco and Hardy 2009). Ciclosporin has also been described for successful management of chronic IMTP refractory to prednisolone in three dogs, although a fourth dog died from systemic aspergillosis that may have been precipitated by immunosuppression (Cook and others 1994).

Acquired myasthenia gravis

Key point This disease has potential for spontaneous remission in the absence of immunosuppressive therapy (Shelton and Lindstrom 2001).

Pathophysiology AMG is caused by autoantibody-mediated blockade and destruction of nicotinic acetylcholine receptors (AchRs; Lindstrom and others 1988), and detection of circulating AchR autoantibodies (>0·6 nmol/l) by radioimmunoassay constitutes the gold standard for diagnosis in dogs and cats. Ninety-eight percent of dogs with generalised AMG are seropositive (Shelton 1998a) with prevalence of antibodies against other muscle determinants under investigation (Shelton 1998b). Two unique aspects of the pathophysiology of AMG merit close attention:

1. AMG is frequently associated with other immune-mediated and neoplastic diseases, in particular, thymoma and hypothyroidism (lymphocytic thyroiditis). Clinicians should be mindful of this during a diagnostic work-up and if treatment response is suboptimal. Reasons for these disease associations are under investigation. In human thymoma, complex defects in intracellular signalling, expression of MHC class II molecules and polymorphisms in immunoregulatory genes within the thymus may lead to failure of central tolerance to AchR antigens and development of myasthenia gravis (Strobel and others 2008). Development of non-thymomatous AMG in mice correlates with polymorphisms in the promoter region of the gene controlling promiscuous expression of AchRs in the thymus. Defects in the autoimmune regulator gene, which controls promiscuous expression of numerous non-thymic antigens in the thymus could account for autoimmunity affecting multiple organs, but it is not understood why certain combinations of organ-specific autoimmunity coexist in people (Kyewski and Taubert 2008). In the case of hypothyroidism and AMG, it has been suggested that the close embryological origins and proximity of the thymus and thyroid gland could lead to thymic myoid cells bearing AchRs being present in the thyroid making the thyroid gland vulnerable to "collateral damage" in immune responses against AchRs (Dewey and others 1995).
2. The natural course of AMG in the absence of thymoma is characterised by an initial strong immune response to AchR, followed by a rapid sustained remission during which AchR autoantibody titres return to normal. This would be compatible with transient exposure to an immunogen (e.g. an infection) resulting in an immune response to AchR that is insufficiently durable to sustain chronic autoimmunity (Shelton and Lindstrom 2001). Dogs that survive the first month following onset of clinical signs routinely go into remission, and a newly diagnosed patient may only require anticholinesterase drugs to enhance neuromuscular transmission and supportive care until remission occurs (Shelton 2002). When a dog responds poorly to such supportive methods and there are no signs of spontaneous remission, or the decline in AchR antibodies that normally signals remission, it seems logical to consider additional therapy with immunosuppressive agents (an approach that is widely employed in management of human AMD; Juel and Massey 2005). However, interpretation of the efficacy of immunosuppression is fraught with difficulty without an appropriate control group of AMD patients not treated with immunosuppressive agents.

Therapy There are no prospective controlled studies evaluating immunosuppressive drug therapy for AMG. Some more recent reports of successful immunosuppressive therapy cite the accelerated timing of clinical and immunological remission compared with the expected timing of spontaneous remission as evidence for drug efficacy. The propensity for myaesthenic dogs to develop aspiration pneumonia is of particular concern when using these agents.

Glucocorticoid therapy for AMG has been described in the dog (Van Heerden and Van Schouwenburg 1983, Maddison and others 1984). Glucocorticoid side effects (worsening of muscle weakness early in the course of therapy, polyuria, polydipsia and polyphagia in patients that may be dysphagic) are a significant concern, prompting the suggestion for use of anti-inflammatory rather than immunosuppressive doses (Shelton 2002). The use of azathioprine has been described as monotherapy or in combination with glucocorticoids in five dogs (Dewey and others 1999). Dewey and others (1997) also reported immunosuppressive therapy (8 of 25 dogs received glucocorticoids and/or azathioprine) to have a significant positive effect on survival in AMG. MMF was reported to induce clinical remission of AMG in a single dog when added to an existing immunosuppressive regime (azathioprine), with relapses occurring when MMF therapy was withdrawn (Dewey and others 2000). MMF has also been used as a rescue agent in three dogs with severe, generalised AMG (two of these dogs received other immunosuppressants; Abelson and others 2009). In a small retrospective study, dogs treated with MMF in addition to pyridostigmine had a higher 1-year survival rate, but this difference was not statistically significant (Dewey and others 2008) and more recent retrospective observations by the same group did not support routine use of MMF for treatment of AMG in the dog (Dewey and others 2010). Ciclosporin therapy

appeared effective in management of two cases of canine AMG following deterioration when receiving glucocorticoid therapy. The authors attributed remission to the ciclosporin because remission was more rapid than would be expected spontaneously (Bexfield and others 2006). Where thymoma is present, complete removal of it is expected to result in resolution of myasthenia gravis (Shelton 2002) but this has not been assessed in a large study.

Glomerulonephritis

Key point Immunosuppressive therapy is currently of no proven benefit for management of canine glomerulonephritis (GN) and may be detrimental.

Pathogenesis Canine GN is generally considered an immune complex-mediated disease. True autoimmune GN with antibodies formed against native glomerular antigens is rarely recorded in dogs. Circulating immune complexes may be deposited in the glomerulus or form in situ as a result of deposited foreign antigen. The location of the immune complex within the glomerulus influences the pattern of histological change, with membranoproliferative, membranous and mesangioproliferative forms documented. Detection and elimination of any neoplastic, infectious or inflammatory condition that could contribute to immune complex formation is the first crucial step in management of GN. Although a number of specific infectious agents can induce glomerular disease, the best documented example is the glomerulonephropathy that occurs as part of canine visceral leishmaniosis. Protein-losing nephropathy with chronic renal failure is cited as the most common cause of death of affected dogs (Saridomichelakis 2009). If the antigen source can be eliminated, functional (if not histological) renal lesions may lessen or resolve over time. In a review of 137 dogs with GN, 43% had no identifiable underlying disease (Cook and Cowgill 1996). The hallmark of glomerular damage is persistent proteinuria with inactive urine sediment (Grauer 2005). There is strong evidence in laboratory animals and man that proteinuria itself becomes a mediator of progressive renal injury (Grauer 2005). Proteinuria is linked to mortality in dogs with naturally occurring renal disease (Jacob and others 2005). Immune complex deposition triggers events that are implicated in progression of glomerular disease: complement and platelet activation, endothelial damage and activation of the renin-angiotensin-aldosterone system, with development of intraglomerular and systemic hypertension (Grauer 2005). These observations have shaped the following treatment recommendations for GN in dogs:

- Reduce proteinuria [using angiotensin-converting enzyme (ACE) inhibitors, modest dietary protein restriction and control of systemic and intraglomerular hypertension]
- Reduce platelet activation (using low dose aspirin, 0·5 mg/kg q 12 to 24 hours)
- Control inflammation (using dietary n-3 fatty acid supplementation)

Efficacy of ACE inhibitor therapy in reducing the magnitude of proteinuria in canine idiopathic GN has been shown in a randomised placebo-controlled study (Grauer and others 2000). Theoretically, low-dose aspirin may reduce the likelihood of pulmonary thromboembolism, an often fatal and poorly understood sequel to proteinuria.

Immunosuppressive therapy The standard of care in human patients centres on obtaining detailed histological characterisation of the lesion. This involves a combination of light microscopy, immunofluorescence and electron microscopy. Based on existing studies, this information yields vital diagnostic and prognostic information such as likelihood of spontaneous remission or likelihood of a favourable response to immunosuppressive therapy. There may be types of glomerular disease in dogs that would benefit from immunosuppressive therapy but veterinarians are often reluctant to perform renal biopsies due to a perception that the results are unlikely to alter the treatment prescribed. This situation will not change unless greater numbers of renal biopsies are procured and analysed by the above techniques. Better characterisation of the natural history of different types of GN will form the basis for prospective studies. Improved standardisation of histological assessment and increased commercial availability of immunofluorescence and electron microscopy should aid recruitment [the International Renal Interest Society and the World Small Animal Veterinary Association (WSAVA) Renal Standardization Group are forwarding these goals]. Currently, biopsies of canine kidney may provide very limited information about prognosis or disease associations, e.g. IgA deposition in dogs with mesangioproliferative GN in Japan has been associated with enteric and hepatic disease (Miyauchi and others 1992). Biopsies also provide information regarding the extent of concurrent tubulointerstitial disease and may assist in ruling out pyelonephritis.

For the moment, there are only two studies evaluating potential benefit of immunosuppressive therapy for naturally occurring idiopathic GN in dogs. In a small prospective placebo-controlled study, ciclosporin offered no benefit in reducing proteinuria (Vaden and others 1995). In a retrospective study, glucocorticoid use appeared detrimental, leading to azotemia and worsening of proteinuria (Center and others 1987).

Inflammatory bowel disease

Key points There is little evidence that IBD is a true autoimmune disease. Manipulation of exogenous antigens in contact with the mucosa and controlling the inflammatory response are important components of treatment.

Pathogenesis The pathogenesis of IBD is complex and genetic background is undoubtedly important, but it is included in this section as an example of an aberrant immune response at a large mucosal surface where exogenous (luminal) rather than endogenous antigens are likely to have an important role in development and perpetuation of the condition. The mechanisms behind this process are poorly understood and the subject of many ongoing studies. A number of controversies surround and have impeded our understanding of IBD. Until recently, a fundamental problem in studying this condition was the lack of consensus regarding what constitutes a clinical and histological diagnosis of IBD and grading the severity of the condition. These problems may have been overcome by the development

of the Canine IBD Activity Index (Jergens and others 2003), which was subsequently adapted to develop the Canine Chronic Enteropathy Clinical Activity Index (Allenspach and others 2007) and by the recent publication of the WSAVA histopathological standards for diagnosis of gastrointestinal inflammation in dogs and cats (Day and others 1998) and the American College of Veterinary Internal Medicine Consensus Statement on IBD by the same group (Washabau and others 2010). Most dogs with primary idiopathic IBD [as opposed to the related disorders of dietary hypersensitivity or antibiotic-responsive diarrhoea (ARD)] respond to glucocorticoid therapy. Clinical improvement in steroid-responsive IBD is often not accompanied by improvement in histopathological lesions (Garcia-Sancho and others 2007, Schreiner and others 2008), although this issue is yet to be assessed using the WSAVA histopathological standards.

Therapy Treatment of canine IBD remains largely empirical due to lack of understanding of the pathogenesis and lack of controlled trials (Craven and others 2004). Prospective identification of dietary sensitivity by laboratory testing is generally not possible and dietary trials are often abortive due to the time, expense and owner frustration at apparent lack of progress when trialling more than one diet is desirable. Dogs with IBD and serological evidence of IgG and/or IgE against food antigens showed poor response to dietary modification when the antigens identified by the test were eliminated from the diet (Guilford and others 2001, Foster and others 2003). Hence, those studies that have been performed on "IBD" therapy are also affected by issues relating to definition of the disease. Although IBD, ARD and dietary hypersensitivity may be considered distinct entities, there is in reality, clinical overlap between the conditions and some authors would define "IBD" as inclusive of cases where primary management was achieved by dietary manipulation.

A significant proportion of dogs with "IBD" have been reported to be refractory to glucocorticoid therapy, but these populations may have included diet-responsive patients. A prospective uncontrolled trial showed a clinical improvement in 12 of 14 such dogs with "IBD" in response to ciclosporin (Allenspach and others 2006a,b). Mucosal expression levels of the drug efflux pump P-glycoprotein may be of value in predicting response to glucocorticoid therapy (Allenspach and others 2006b). Use of glucocorticoids with increased topical activity and fewer systemic effects (budesonide) may be appropriate for dogs experiencing unacceptable side effects of prednisolone (Battersby and others 2007). Other immunosuppressive agents commonly employed in cases not responding to prednisolone alone include azathioprine in dogs, but studies documenting efficacy are lacking. A recent prospective randomised non-placebo-controlled study showed that the combination of prednisone and metronidazole offered no benefit compared with prednisone alone for induction therapy of canine IBD (Jergens and others 2010).

FUTURE DIRECTIONS

This review has covered approaches to medical immunoregulation in canine immune-mediated disease as they are currently practiced. Although these drugs may be used to successfully manage the clinical signs of disease and may in some cases induce remission from disease, the agents are less than satisfactory from the immunological perspective. The drugs described above generally have very broad effects on the immune system and even those with more selective action on T cells (i.e. ciclosporin) will also affect indirectly on B cell function. The non-specific immunosuppression mediated by these agents underlies the susceptibility to secondary infection that is an important side effect of the drugs.

Given the current knowledge of immunological mechanisms described above, it would be far more satisfying, both clinically and immunologically, to develop new technologies that might target specific aspects of a deleterious immune response. This is now widely practiced in human medicine where there is an array of licensed recombinant human cytokines that when delivered locally or systemically can modulate the immune response. These products are complemented by a range of monoclonal antibodies specific for cytokines and other key immunological molecules that when given intravenously will target only those molecules and neutralise or block their effects (Day 2005). The first stages of this new style of medical therapy are now appearing in companion animal medicine. Canine recombinant IFN-γ is now licensed in Japan (Interdog™; Toray Industries, Tokyo, Japan) for the management of canine atopic dermatitis by rebalancing the activity of Th1 and Th2 lymphocytes in this disease (Yasukawa and others 2010). Clinical trials underway in human medicine are beginning to explore the application of even more specific immunomodulation. Administration of peptide fragments of autoantigens or allergens across mucosal surfaces (e.g. by intranasal or sublingual delivery) appears able to expand the activity of Treg that counteract the pathogenic effects of autoreactive or allergenic Th1 and Th2 cells (Larche and Wraith 2005). A small clinical trial on the use of a peptide vaccine (administered subcutaneously) has been performed in pet dogs suffering from spontaneous myaesthenia gravis (Galin and others 2007). The application of such technology to veterinary medicine requires considerable further research but remains an important goal.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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