REVIEW

A review of anaemia of inflammatory disease in dogs and cats

S. Chikazawa*,[1] and M. D. Dunning†

*Department of Small Animal Internal Medicine, School of Veterinary Medicine, Kitasato University, 23-35-1 Towada, Aomori 034-8628, Japan
†Department of Medicine and Health Sciences, School of Veterinary Medicine, University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD
[1]Corresponding author email: chikazaw@vmas.kitasato-u.ac.jp

Anaemia of inflammatory disease is a common cause of anaemia in routine veterinary practice. It is most often mild to moderate, normocytic, normochromic and non-regenerative. Shortened red cell life span, inhibition of iron metabolism and impaired bone marrow response to erythropoietin all contribute to its development. Although anaemia of inflammatory disease is a well-known cause of anaemia in dogs and cats, there is a lack of epidemiological information because specific diagnostic criteria have not been established in veterinary species. Anaemia of inflammatory disease is associated with a poor outcome in various disease states in human medicine; however, its clinical significance and treatment in veterinary medicine are not well understood. This review article describes anaemia of inflammatory disease in dogs and cats and considers its potential significance.

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INTRODUCTION

Anaemia is commonly diagnosed in small animal practice and arises from a number of different causes. Anaemia of inflammatory disease (AID) is one of the most common causes in dogs and cats (Fry 2010). It is characteristically non-regenerative, of mild-to-moderate severity and is associated with various chronic disorders including infectious, inflammatory, immune-mediated, and neoplastic diseases (Waner & Harrus 2001, Fry 2010). Although this type of anaemia has also been called "anaemia of chronic disease (ACD)", no consensus exists with regard to terminology. This review hereafter refers to it as "AID" because it is believed that inflammation plays a key role in this type of anaemia. AID is generally characterized by hypoferraemia, a low to normal serum total iron-binding capacity (TIBC), normal or high body iron storage and failure of bone marrow to adequately increase erythrocyte production. The physiological role of AID is thought to be part of the normal immunologic response to limit microbial access to iron (Fry 2010). Although AID has multifactorial causes, the major pathophysiological mechanism is functional iron deficiency. Additionally, the clinicopathological features of AID include a slightly shortened red cell life span, inhibition of iron metabolism and impaired erythropoietin-mediated erythropoiesis in the bone marrow (Fry 2010). However, reliable diagnostic criteria for AID are yet to be established in veterinary medicine.

Chervier *et al.* (2012) reported the causes of anaemia (PCV<37%) in 456 dogs, excluding acute haemorrhage. AID was the second most frequent cause (28·5%) of anaemia in this group of dogs, following cancer-related anaemia (33·1%). In the report, AID was diagnosed in dogs with anaemia displaying inflammation but with no signs of infectious, neoplastic or immune-mediated disease. The authors highlighted as a limitation of the study that AID is nevertheless frequently associated with various inflammatory conditions such as infectious and neoplastic diseases. In such cases they emphasised that it is not possible to determine the precise mechanism of anaemia.

To our knowledge, there are no reports describing the epidemiology of AID in cats. This article reviews the current information on AID in dogs and cats and considers future challenges for diagnosing and treating this condition.

AETIOLOGY AND PATHOGENESIS OF AID

In the early part of 20th century, in human medicine, it was accepted that anaemia developed in association with inflammation

in patients with rheumatoid arthritis (Collins & Liverp 1935). This was also identified in various acute and chronic infections such as endocarditis (Pepper 1927), osteomyelitis, meningococcaemia, cellulitis, empyema and pyelonephrosis (Saifi & Vaughan 1944). Cartwright *et al.* (1946b) described the morphological features of the anaemia that developed in such patients as frequently normocytic and normochromic, although in some cases it could be mildly microcytic and hypochromic. These authors also demonstrated that the anaemia resulted from suppression of iron metabolism and diversion of iron to the bone marrow, rendering it unavailable for haemoglobin synthesis. Subsequent to these observations, numerous animal models have been used to study AID, including dogs (Cartwright *et al.* 1946a, Feldman *et al.* 1981, Chikazawa *et al.* 2013), cats (Mahaffey & Smith 1978, Weiss *et al.* 1983), rats (Lukens *et al.* 1967), mice (Gallimore *et al.* 1991) and rabbits (Karle 1969). These models have used agents (infectious or non-infectious) to induce AID. Mouse models have provided the greatest number of important reports elucidating the pathogenesis of AID. This is because mice have a haematologic and inflammatory system similar to humans (Rivera & Ganz 2009). As described above, current understanding of the pathogenesis of AID is characterised by three main pathophysiological factors: shortened red cell life span, inhibition of iron metabolism and impaired bone marrow response to erythropoietin.

Red cell life span

A moderate reduction in red cell life span has been reported in cats with experimental AID (Weiss & Krehbiel 1983). In human medicine, it is recognised that anaemic patients with active rheumatoid arthritis demonstrate ineffective erythropoiesis and shortened mean red cell lifespan (Dinant & de Maat 1978). This effect has been suggested to have an immunological basis. Macrophages become activated due to various inflammatory cytokines liberated during inflammation, such as tumour necrosis factor (TNF)-alpha (Moldawer *et al.* 1989). This accelerates the clearance of erythrocytes from the circulation via erythrophagocytosis by tissue macrophages. This erythrophagocytosis is accelerated by gamma immunoglobulins coating the red cells; a phenomenon highlighted using a feline model of AID (Singer *et al.* 1986, Weiss & McClay 1988).

Inhibition of iron metabolism

Hypoferremia is observed in AID despite adequate body iron stores, indicating a disturbance in iron metabolism. This change is thought to be part of an important host immunologic response to limit microbial access to iron and results from suppression of gastrointestinal iron absorption and increased iron retention within macrophages involved in inflammation (Feldman & Kaneko 1981, Fry 2010). Serum iron circulates bound to transferrin (Tf) and this provides the body's iron requirement as part of a semi-closed iron recycling system (Fig 1). The aged red blood cells are phagocytised and destroyed by tissue macrophages and haem-derived iron is released back into the circulation (Delaby *et al.* 2005). The amount of serum iron bound to Tf is a relatively small amount (<1%) of the total body iron store and it is constantly used and recycled (Hentze *et al.* 2004). Therefore, once iron release from macrophages is blocked, serum iron concentration rapidly decreases and as a result, the supply of iron for haemoglobin synthesis in the bone marrow is reduced and erythropoiesis is suppressed. This is an important mechanism triggering the development of AID.

The bioactive form of the type II acute-phase protein hepcidin, is comprised of 25 amino acids (hepcidin-25) and is induced by iron overload and inflammatory cytokines such as interleukin (IL) -6 or IL-1 (Nemeth *et al.* 2003, Inamura *et al.* 2005, Ganz & Nemeth 2011). Hepcidin is a hormone predominantly produced by hepatocytes and generally accepted as the key mediator of AID (Fry 2010). It binds to ferroportin which is the only known cellular iron exporter, mainly expressed on the surface of intestinal enterocytes, macrophages, hepatocytes and placental cells (Abbound & Haile 2000, Nemeth *et al.* 2004b). After binding, ferroportin is internalised and degraded, blocking iron absorption and macrophage iron recycling (Nemeth *et al.* 2004b). Overexpression of hepcidin in transgenic mice leads to severe iron deficiency (Nicolas *et al.* 2002a), although hepcidin gene knockout mice do not develop hypoferremia in turpentine-induced sterile abscesses (Nicolas *et al.* 2002b). Turpentine has been used to elicit a local inflammatory response in animal models of AID for more than 50 years (Rivera & Ganz 2009). In addition, IL-6 gene knockout mice do not show hypoferremia or positive hepcidin gene expression (Nemeth *et al.* 2004a), suggesting that IL-6 plays a central role in hepcidin expression and the existence of an IL-6-hepcidin axis (Fig 2).

In a recent study, haemoglobin concentration and erythrocyte number were decreased in IL-6 gene knockout mice with sterile abscesses. However the significant decline in erythrocyte number in hepcidin gene knockout mice, indicates hepcidin independent mechanisms exist that impair erythrocyte response during inflammation (Langdon *et al.* 2014). In dogs, hepatic expression of the hepcidin gene is decreased by nutritional iron deficiency (Fry *et al.* 2009) and increased during turpentine-induced inflammation (Chikazawa *et al.* 2013). However, during experimentally induced long-term inflammation in dogs using repeated subcutaneous administration of turpentine, hepatic hepcidin gene expression only increased in the initial phase of the experiment and normalised during the later period, though the anaemia and hypoferremia persisted (Chikazawa *et al.* 2013). Similar observations were noted in a mouse AID model, which used repeated administration of turpentine (Sun *et al.* 2006). These results indicate that while hepcidin is an important mediator of AID, other mechanisms are involved in development and maintenance of AID.

Bone marrow function

Bone marrow erythropoiesis is impaired in AID. This defective erythropoiesis results from several inflammatory cytokines inducing impaired erythropoietin secretion and/or response along with direct toxicity on the erythroid precursor cells.

It has been reported that IL-1 and TNF-alpha inhibit erythropoietin gene expression in hypoxia-induced models of erythropoietin secretion, using a human hepatoma cell line (Faquin *et al.* 1992). Additionally, IL-1 and TNF-alpha suppress erythropoietin

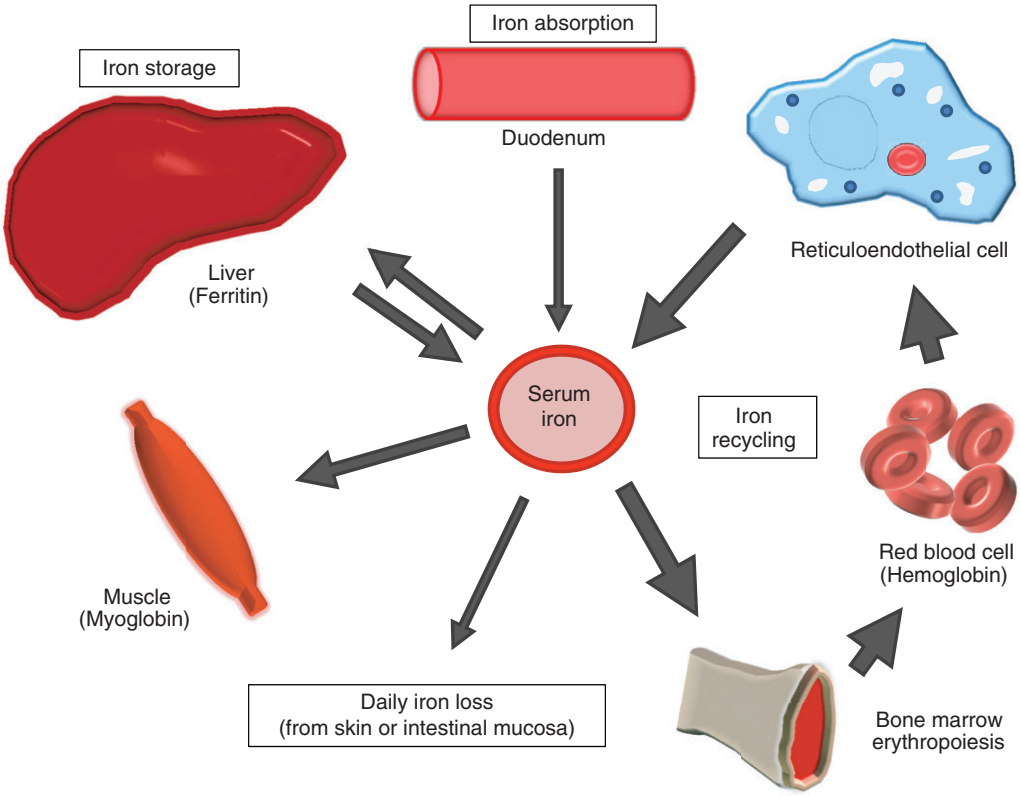

FIG 1. Overview of mammalian iron metabolism. The amount of iron absorption from food is extremely small (1 to 2 mg/day in human adults) and is approximately equal to the daily loss of iron. Most of the iron required for haematosis is derived from haemoglobin recycled from aging erythrocytes through erythrophagocytosis performed by reticuloendothelial cells. Excess iron is primarily stored in the liver as ferritin and released into the circulation as needed

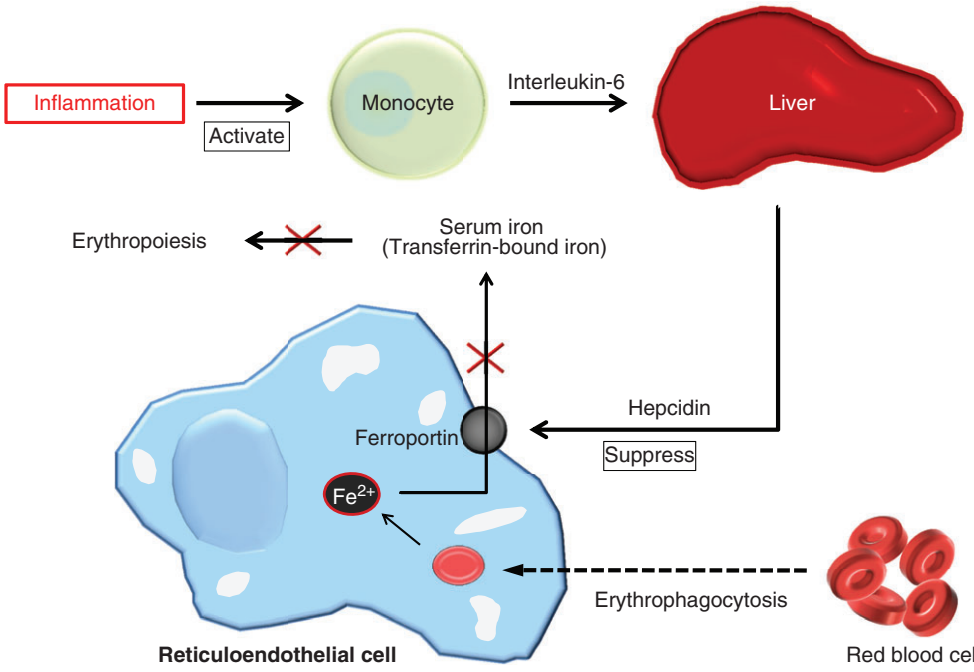

FIG 2. Pathophysiological mechanisms of the interleukin-6-hepcidin axis underlying anaemia of inflammatory disease. Monocytes activated by inflammation promote hepcidin gene expression in the liver through interleukin-6 production. Hepcidin is released into the blood and inhibits the function of ferroportin, which is an exporter of iron for reticuloendothelial cells. Ferroportin plays a key role in iron metabolism and rapidly reduces serum iron concentration. A decrease in serum iron concentration inhibits erythropoiesis and induces anaemia

gene expression in the rat kidney (Jelkmann 1998). These results suggest that several inflammatory cytokines are associated with negative regulation of erythropoietin in AID. However, the influences on the release of erythropoietin in clinical AID are less clear. In humans, serum erythropoietin concentration in anaemic patients with chronic diseases is significantly lower than in patients with other causes of anaemia (Bear *et al.* 1987, Boyd & Lappin 1991). However, there are reports that the serum erythropoietin concentration is increased in patients with rheumatoid arthritis (Nielsen *et al.* 1990). In veterinary medicine, serum erythropoietin concentration in an experimentally-induced feline AID model was either within the reference interval or was slightly increased (Weiss *et al.* 1983). Therefore these slight differences in erythropoietin concentration may be less important for development of AID than the bone marrow's response to erythropoietin (at least in cats and in some comorbid diseases in humans). To the authors' knowledge, there are no reports describing erythropoietin concentrations in dogs with AID.

In AID, proliferation and differentiation of erythroid precursors is impaired as a result of altered responsiveness to erythropoietin (Weiss & Goodnough 2005). Underlying mechanisms may include cytokine-mediated induction of apoptosis, down-regulation of erythropoietin receptor expression on erythroid progenitor cells, reduced expression of other pro-haematopoietic factors such as stem-cell factor and reduced numbers of erythroid colony-forming units (Taniguchi *et al.* 1997).

LABORATORY FEATURES AND DIAGNOSIS OF AID

Since AID is in fact a "functional" state of iron deficiency, it shares some clinical characteristics with true iron deficiency anaemia (IDA), including hypoferremia and a variety of changes in erythrocyte morphology (Waner & Harrus 2001). Pure IDA is characterised by a decrease in MCV and MCHC, moderate to marked thrombocytosis and an increase in red blood cell distribution width. In addition, characteristic changes on the blood film include a large area of central pallor, poikilocytosis including keratocytes and schistocytes (Weiss 2010). The clinical value of reticulocyte indices, particularly the haemoglobin content and mean reticulocyte volume have been shown in dogs with experimentally induced nutritional and clinical iron deficiency (Steinberg & Olver 2005, Fry & Kirk 2006, Schaefer & Stokol 2015). However, because there is no information about how these parameters change in the process of IDA, especially acute iron deficiency and the effect of underlying diseases, the diagnosis remains difficult.

Differentiating between AID and IDA may be difficult but iron parameters and characteristic erythrocyte morphology may help in making a diagnosis. TIBC reflects the Tf concentration in serum (Harvey 2008). In dogs, TIBC is reported to be slightly increased in experimentally induced nutritional iron deficiency (Fry & Kirk 2006) and decreased in turpentine-induced AID (Chikazawa *et al.* 2013). Since Tf is one of the negative acute-phase proteins, along with albumin (Cerón *et al.* 2005), inflammation is associated with a low TIBC. However, malnutrition in dogs is also reported to cause a reduction in plasma Tf concentration (Nakajima *et al.* 2014). In addition, clinical IDA in dogs is generally associated with a normal TIBC (Weiser & O'Gray 1983). Therefore, TIBC has limited value as a parameter to distinguish between IDA and AID.

One of the important pathophysiological differences between AID and IDA is the total body iron storage. This is normal or increased in AID but is decreased in IDA (Naigamwalla *et al.* 2012). Since serum ferritin concentration and liver and bone marrow iron content reflect body iron storage (Gale *et al.* 1963, Worwood 1990, Angelucci *et al.* 2000), these are useful markers for diagnosis. Although measurement of serum ferritin concentration is a simple method, it also varies independently of the body iron storage in inflammatory conditions (Harvey 2008). Therefore, because serum ferritin concentration does not always reflect the body iron storage, it should not be used alone to differentiate between IDA and AID. Other indicators of total body iron storage include staining bone marrow samples and liver biopsies for qualitative iron determination; however, these methods are invasive and rarely performed in routine primary care veterinary practices. An important dilemma exists in the diagnosis of complicated AID in dogs and cats. No information exists in veterinary medicine about the changes in iron parameters in patients with coexistent AID and IDA. In human medicine, AID and IDA are often categorised based on clinical findings using the parameters described above plus serum Tf receptor (sTfR) concentrations. The sTfR is strongly and positively correlated with total body iron storage (Cook *et al.* 2003) and is primarily produced by bone marrow erythroid precursors according to their iron requirement (Skikne 2008). The sTfR concentration is less likely to be influenced by chronic disease than serum ferritin and is increased in IDA, but not in AID (Berlin *et al.* 2011, Braga *et al.* 2014). However, to the authors' knowledge, there are no reports measuring sTfR concentration in dogs and cats with AID.

A combination of clinical history, physical examination and routine laboratory tests are therefore important for the diagnosis of AID. However, since underlying diseases lead to a variety of other haematologic changes, a final diagnosis of AID should be made after other causes of anaemia such as nutritional deficiencies, hypothyroidism, myelodysplasia, drug reactions, chronic kidney disease and haemorrhage are ruled out.

MANAGEMENT OF AID

Although AID is one of the most common causes of anaemia, it is rarely severe enough to require treatment (Waner & Harrus 2001). Importantly, and where possible, treatment of the underlying disease triggering the inflammation should be introduced before any specific treatment for the anaemia is initiated. In some chronic diseases, such as malignant neoplasia, curative treatment is often impossible (McCown & Specht 2011) and in such cases it is unclear (in veterinary species) whether treating the AID improves the outcome. One review by Miller *et al.*

(2009) reported a PCV of less than 35% at initial presentation (before chemotherapy) in 84 dogs with lymphoma was associated with short survival time. It is possible that the anaemia in these dogs was associated with AID. If so, this suggests that AID may influence the prognosis in canine lymphoproliferative disease. In human medicine, anaemia, including AID, affects morbidity, mortality, and the quality of life in affected patients (Nissenson *et al.* 2003). Although treatment of the underlying disease is the best therapeutic approach for AID, this may not be possible and alternative strategies may be required. These include blood transfusions, parenteral iron therapy and administration of recombinant human erythropoietin (Cullis 2013).

FUTURE CHALLENGES FOR AID

We have emphasised in this article that in order to improve the diagnosis of AID it is important to establish a standard method for differentiating it from IDA and from AID with concomitant IDA. We have highlighted the importance of research methods that evaluate body iron storage in addition to the conventional diagnostic methods such as complete blood count, red blood cell morphology, iron parameters and reticulocyte indices. However, as it is difficult to justify invasive examinations such as biopsies of the liver or bone marrow in primary care practice, the development of alternative non-invasive methods is desirable. In human medicine, serum hepcidin measurement is one particular marker used to aid with differentiation between AID, AID with concomitant IDA, pure IDA and acute inflammation associated anaemia (Cheng *et al.* 2011). It is therefore considered of value in dogs and cats to validate assays measuring serum hepcidin together with sTfR.

In summary, since it is difficult at present to reach an unequivocal diagnosis of AID in dogs and cats the prevalence, relationship with prognosis and clinical significance of treatment all remain unknown. The current publications on AID in dogs and cats provide limited details of the mechanism and its impact on the underlying disease states in which it occurs. It is hoped that with future work on this subject, information will emerge to help identify its real impact and the optimum management strategies. In the future, once a reliable diagnostic method for AID is established, the epidemiology will become clear along with the clinical significance of treatment. It is hoped that this review has provided important background information on the subject and will stimulate further work to characterise AID in both dogs and cats.

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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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