LESSON LEARNED: IMMUNE-MEDIATED HEMOLYTIC ANEMIA IN DOGS

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Primary and Secondary Immune-Mediated Hemolytic Anemia (IMHA)

Immune-mediated hemolytic anemia (IMHA) is a type II immune reaction in which circulating red blood cell (RBC) destruction is antibody-mediated (cytotoxic) (Day 2014, Tizard 2013, Chabanne 2006, Day in Fieldman *et al.* 2000). Antibody attachment to erythrocyte surface depends on two main causes:

1. The majority of **primary IMHA** are **idiopathic** or associated with other autoimmune diseases in the autoimmune form of the disease, antibodies (auto-antibodies) recognize a self-antigen of the erythrocyte membrane. Hemolysis dominates the clinical picture in the absence of any other coexisting disorder. Autoimmune hemolytic anemia may occur as a single clinical entity (idiopathic), may be recognized concurrently with autoimmune thrombocytopenia (Evans syndrome), or maybe part of a multisystemic autoimmune disease like systemic lupus erythematosus (SLE). The development of autoimmunity results from a failure of the normal control mechanisms of the immune system. Autoimmune diseases are multifactorial disorders in which clinical expression relies on the presence of an optimum array of predisposing factors. Genetic factors are key determinants of disease susceptibility that explain breed or familial predispositions. Other predisposing factors must be important: hormonal background, age, and environmental factors (infectious agents, drugs and chemicals, etc.). The aim in the treatment of IMHA as with all autoimmune diseases is to manipulate (to down-regulate or suppress) the immune response that causes the disease so that the process is reduced or abolished (Berentsen 2011, Petz and Garratty 2004, Gehrs and Friedberg 2002).
2. In **secondary IMHA**, accompanying and complicating an underlying disease. The surface of the red blood cells becomes altered by an underlying disease process or a toxin. Antibodies have a specificity for a foreign antigen (an infectious agent or a drug)

that is associated with the red blood cell surface, or for a neo-antigen, which is red blood cell determinant modified by a drug, an infectious agent or secondary to a neoplastic phenomenon. Immune complexes can also be adsorbed at the red blood cell surface. In this case, red blood cell destruction is due to bystander hemolysis as the causative antibody is not specific to the normal red blood cell. The dog's immune system recognizes the altered red blood cells as 'foreign' invaders that must be destroyed. Secondary IMHA may be triggered by cancer, infection as seen with *Ehrlichia*, *Leptospira*, blood parasites such as *Babesia*, drug reactions, snakebites, chemicals, toxins, or bee stings. In dogs, neoplasia or cancer appears to be the most common cause of secondary IMHA. Stressful events on the body may trigger IMHA if underlying secondary causes are already present. The prognosis of secondary IMHA is more closely related to the underlying disorder than the hemolytic anemia. Therapy should be directed to the control of this disease, and the management of the hemolytic process itself (Berentsen 2011, Petz and Garratty 2004, Gehrs and Friedberg 2002).

Immune-Mediated Hemolytic Anemia Associated with Intravascular and/or Extravascular Hemolysis

In **intravascular hemolysis**, Immunoglobulin M (IgM**)** antibodies activate complement, and destroy red cells (Tizard 2013). IgM are pentameric autoantibodies able to dramatically fix complement and to a lesser extent C3d-mediated extravascular lysis (mainly in the liver). Their optimal temperature of reaction is 4°C, and thus they are responsible for the cold forms of IMHA (Berentsen 2011, Petz and Garratty 2004). However, the thermal amplitude of IgM autoantibodies ranges from 0 to 34 °C, and those with a thermal activity close to physiological temperatures (warm IgM) are the most harmful autoantibodies, able to cause severe forms of IMHA with a reported mortality rate of about 20% (Garratty and Arndt 2014). This results in hemoglobinemia, hemoglobinuria, icterus, and very severe anemia. Affected dogs are anemic, weak, and possibly jaundiced. Kupffer cells in the liver or macrophages in lymph nodes preferentially remove red cells with a complement on their surface, so these dogs develop hepatomegaly and lymphadenopathy (Tizard 2013).

The most frequent autoantibodies against RBCs are IgG, which mainly determines **extravascular hemolysis** through the reticuloendothelial system (spleen and to a lesser extent

liver). The IgG subclass influences the degree to which these antibodies shorten RBC survival: IgG1 is the most commonly encountered subclass and together with IgG3 shortens the half-life more efficiently than IgG2 and IgG4. Autoantibodies of this class are mostly directed against epitopes of the Rh system. They generally react at 37°C and, therefore, are responsible for the warm forms of IMHA (Petz and Garratty 2004). Immunoglobulin G is able to activate the complement cascade, with the exception of IgG2 and IgG4 subclasses. Immunoglobulin A autoantibodies are generally associated with IgG and only rarely reported as the sole causative agent of IMHA (Barcellini 2015).

Correlation of IMHA with Unnecessary Vaccination

The veterinarian widely believed that the prevalence of autoimmune disease in dogs has risen because of excessive use of potent vaccines (Tizard 2013). Although there is little doubt that this can happen in individual patients, it has proven difficult to define the immunological mechanism that might explain the association (Day 2014). A retrospective analysis of the history of dogs presenting with IMHA showed that 15-70 dogs with IMHA had been vaccinated within the previous month, compared with a randomly selected control group in which none had been vaccinated. Dogs with IMHA that developed within a month of vaccination differed in some clinical features from dogs with IMHA unassociated with prior vaccination. Epidemiological studies show an approximately three-fold increase in diagnoses of autoimmune thrombocytopenia, and a two-fold increase in diagnoses of IMHA, in dogs in the 30 days following vaccination, compared with other time periods. The overall incidence of these diseases, however, is low, they can be diagnosed at times not temporally associated with vaccination. Vaccination may therefore serve as a stimulus for these diseases in some dogs, but other, undefined, stimuli must also exist (Tizard 2013).

It is not known whether the vaccine components induced autoantibody production in the dogs in many studies, or whether the macrophages or the immune system were activated by vaccination to destroy red blood cells with pre-existing antibodies on the erythrocyte surface. In drug-induced IMHA, drugs can induce autoantibodies or antibody production against red blood cells that may require the involvement of the drug in the antibody-erythrocyte binding. Although vaccine components are given in small quantities, they contain relatively large amounts of immune-reactive antigens and other components that may remain in the body for extended periods of time. Therefore, vaccination can elicit an immune response days to weeks after inoculation. Drug-induced IMHA resolves with drug withdrawal and has a good vaccine-

associated IMHA, in contrast, has a mortality rate and clinical course more closely resembling idiopathic IMHA (Duval and Giger 1996).

In human comparison, vaccine-associated IMHA has been reported after diphtheria-pertussis-tetanus vaccination in children. Similar mechanisms are proposed for vaccine-induced IMHA as for Immune-Mediated Thrombocytopenia Purpura (IMTP). Surface erythrocyte binding and agglutination were seen when diphtheria and tetanus portions of the vaccine were added to erythrocytes from healthy people and patients with vaccine-associated IMHA. Immunoglobulins eluted off erythrocytes from these patients had antigen specificity for the various components of the diphtheria-pertussis-tetanus vaccine. These findings may support an immune-mediated model, with antibodies formed against erythrocyte membrane-attached vaccine components. In a guinea pig model of IMHA, antibody-coated erythrocyte removal from the circulation was markedly accelerated when the guinea pig's immune system was previously stimulated with bacillus Calmette-Guerin. This evidence suggests that immune stimulation and macrophage activation, such as might be induced by vaccination, could exacerbate pre-existing IMHA.

Four possible mechanisms by which vaccines can induce IMTP (same as with IMHA) have been proposed, but direct evidence is lacking. First, the vaccine virus may inhibit thrombocytosis at the megakaryocyte stage. However, in people, bone marrow aspirates showed increased megakaryocyte numbers and blood smears revealed enlarged platelets suggestive of accelerated thrombopoiesis in these cases. Second, vaccine components may attach to or alter the platelet membrane and elicit an immune surface antigens or cross-reactivity with platelets and vaccine components have not yet been demonstrated. In a similar fashion, vaccine components may combine with proteins to act as haptens and invoke immune complex formation. This appears to be the case in distemper-induced thrombocytopenia in experimentally infected dogs, although the specificity of the platelet-bound antibodies was not determined. Fourth, vaccination may alter the immune response so that normal platelets are removed by an overactive immune Injection with pneumococcal vaccine antigens in mice triggering a polyclonal B-lymphocyte stimulation that can result in IMTP similar to that seen in sepsis-induced IMTP. Experimentally, immune modulatory drugs such as levamisole may reduce the prevalence of thrombocytopenia induced after vaccination for the distemper virus in dogs. Further studies are needed to elucidate the cause of vaccine-induced IMTP (Duval and Giger 1996).

In the dogs, 32 papers mentioned that vaccines could be a trigger for IMHA, of which only 12 papers describe 79 clinical cases with documented temporal associations of ≤30 days between

vaccine administration and IMHA. The types of vaccines given to each patient were not consistently recorded. Three papers provide evidence for a link between vaccination and IMHA in the low range, with IME values between 2.95 and 4.37. No publications that provide high levels of evidence to support an association between vaccination and IMHA were found. Considering the wide practice of vaccination and the lack of conclusive evidence of an association with IMHA, current vaccination strategies generally are safe. Patients should be individually assessed for their own risks and benefits before vaccination. Further studies are needed to determine if and when vaccine-associated IMHA occurs in dogs, and to develop better methods for the diagnosis of vaccine-associated disease (Garden *et al.* 2019).

Surgery Treatment of Splenectomy in Dogs with IMHA

Some practitioners perform splenectomy on dogs that have IMHA with splenomegaly. A splenectomy is a surgical procedure that partially or completely removes the spleen. The spleen is one of the largest lymphatic organs and the only one that is directly connected to the blood-circulatory system. As a filter organ, it strains senescent and/or defective red blood cells out of the circulating blood (Uranus 1995). The spleen has a central role in the pathogenesis of immune-mediated hemolytic anemia (IMHA). It is typically the major site of mononuclear phagocytic system removal of IgG-coated RBCs in patients with IMHA and has an integral role in antigen presentation and autoantibody production (Cohn 2004). Removal of the spleen can eliminate a major contributor to the pathogenesis of RBC destruction in patients with IMHA but is reserved for patients who do not respond to standard immunosuppressive therapy or who experience adverse effects associated with drug therapy (Feldman 1985).

A recent preliminary study (Toll and Aronsohn 2003) in a relatively small group of dogs described the use of early splenectomy to treat patients with acute IMHA and found that dogs treated with glucocorticoids and azathioprine as well as splenectomy within 48 hours of presentation had shorter recovery times and higher survival rates than dogs treated with glucocorticoids and azathioprine alone. Based on these preliminary results, further investigation into early splenectomy is warranted, but they cannot advocate this procedure because of its high risk. Risks associated with splenectomy include complications associated with anesthesia and surgery and an increased predisposition to infectious diseases (bacterial sepsis, blood-borne parasitemia) that would normally be controlled by the spleen (Balch and Mackin 2007)

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