XVIII Congreso de Especialidades Veterinarias
26-27 de Abril de 2019 - Palacio de Congresos- ZARAGOZA

PRIMARY AND SECONDARY IMMUNODEFICIENCIES

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INTRODUCTION

The most challenging area of clinical immunology is the recognition and confirmation of primary inherited immunodeficiency disease. There are around 20 such disorders recognized in particular breeds of dog – but primary (as opposed to secondary) immunodeficiency seems rare in the cat. This lecture will discuss the general approach to diagnosis of immunodeficiency and review some of the more interesting and common canine disorders. These canine disorders are often employed as experimental models for equivalent human diseases, and in this context, astounding success is sometimes achieved therapeutically.

Primary, congenital, inherited immunodeficiency disorders are uncommon in the dog. By contrast, immunodeficiency in adult animals secondary to a wide range of defined causes (e.g. age, drug therapy, chronic neoplastic or infectious disease) is relatively common. A good example of putative secondary immunodeficiency is the state of immune dysregulation that is associated with generalized demodicosis in dogs. It is proposed that *Demodex* mites may produce immunomodulatory factors that inhibit protective immune responses in this disease. Recent studies have demonstrated immune defects in affected dogs, including: (1) inhibition of $CD4^+$ T cells (by apoptosis or immune exhaustion), (2) increased expression of Toll-like receptor (TLR)-2 and decreased expression of TLR-4 and -6 on circulating leucocytes, and (3) increased circulating TGF-β and IL-10 with decreased TNF-α.

The primary immunodeficiencies reflect a developmental block at one or more levels of maturation of the immune or haemopoietic systems. A defect at the level of stem cells will give rise to a more profound clinical syndrome than for example, a selective inability to produce immunoglobulin of a single class. Primary immunodeficiency will often be recognised within litters of animals after the protective effect of maternal immunoglobulin has been lost (12–15 weeks of age). Most canine immunodeficiencies are breed-related. The occurrence of chronic recurrent infections, infection at multiple sites, infections with environmental saprophytes or illness after modified live virus vaccination in susceptible animals of this age should trigger investigation of possible immunodeficiency.

DIAGNOSIS OF IMMUNODEFICIENCY

The diagnosis of immunodeficiency should progress through stages of complexity. Basic characterisation of the problem may include routine haematological examination (specifically assessing leucocytes), assessment of serum proteins, culture and identification of pathogens, and full necropsy examination of any affected animals (i.e. within a litter) that die during the investigation. The necropsy examination should pay specific attention to lymphoid organs and histological examination of thymus, spleen, lymph node, bone marrow and gut should be performed if possible. Analysis of pedigree data should also be performed.

The ideal secondary stage of investigation would include full screening of the quantity and functional capacity of the various components of the immune system, including the humoral, cell-mediated and phagocytic arms. In theory, a very detailed examination of the canine immune system is now possible and a wide range of techniques has been validated in a research setting. In practice, such tests are rarely available on a commercial basis to veterinarians in practice. It is generally possible to have the serum concentration of the major immunoglobulin classes (IgG, IgM and IgA) assessed in the dog, and commercial laboratories have increasing access to assessment of blood lymphocyte subpopulations (e.g. CD4 and CD8 T lymphocytes, CD21 B lymphocytes) by flow cytometry. Unfortunately, the more relevant tests of lymphocyte proliferative response and neutrophil/macrophage chemotaxis, phagocytosis and killing assays are less accessible. In selected canine immunodeficiencies (X-linked severe combined immunodeficiency, cyclic haematopoiesis, canine leucocyte adhesion deficiency, lethal acrodermatitis, trapped neutrophil syndrome), the precise molecular defect has been characterised, and commercially available molecular tests are now often

available. Molecular tests may also be used to confirm the presence of feline leucocyte adhesion deficiency and the immunodeficiency of Birman cats.

IMMUNODEFICIENCY DISEASES IN THE DOG

The major primary immunodeficiency diseases of the dog are summarised below. Some of these reports refer to limited episodes in specific groups of dogs kept for research purposes, and the defects are not widespread in the breeds. In many cases the immunological defects and their mode of transmission have been poorly characterized.

The most widely recognised canine (and human) immunodeficiency is selective deficiency of immunoglobulin A (IgA). In both species this appears not to be an absolute lack of IgA due to a mutation in the gene encoding the immunoglobulin alpha chain, but a relative deficiency in concentration of this immunoglobulin in serum or at mucosal surfaces. IgA deficiency has been reported in a number of breeds and a recent study from Sweden has highlighted the shar pei, hovarwart, Nova Scotia duck tolling retriever, Norwegian elkhound, bearded collie, German shepherd dog, Belgian shepherd dog, golden retriever, bull terrier and rottweiler as affected. As in humans, canine IgA deficiency has been clinically associated with a range of infectious, inflammatory, allergic and autoimmune diseases.

Most research into canine IgA deficiency has been undertaken in dogs of the German shepherd breed. Our own investigations of German shepherd dogs (GSDs) with inflammatory enteropathies have shown that these dogs may have low concentrations of IgA in the intestinal lumen (by in-vitro secretion in explant cultures, but not in faecal IgA concentration) despite having normal numbers of IgA secreting plasma cells in the intestinal mucosa, and normal serum concentrations of this molecule. There are no defects in the ability of GSDs to express the IgA alpha chain gene, or the genes encoding molecules involved in the transport of IgA across the intestinal epithelial barrier. Molecular studies have identified four genetic variants of canine IgA, and selective usage of one particular variant occurs in GSDs.

In contrast, other groups have reported reduced faecal IgA concentration in GSDs or reduced faecal IgA and mucosal IgA^+ plasma cells in dogs with inflammatory enteropathy.

Recent studies of Irish wolfhounds with chronic recurrent rhinitis and/or bronchopneumonia have also suggested a role for IgA deficiency in the pathogenesis of the disorder. Affected dogs may have low serum IgA, but the concentration of IgA within bronchoalveolar lavage fluid is normal or raised. There are also lymphocyte subset abnormalities documented within this lavage fluid, and poor lymphocyte response to mitogenic stimulation is described.

English bull terriers with ‘lethal acrodermatitis’ have been shown to have selective deficiency of serum IgA concentration. In addition, affected dogs have poor responsiveness of T lymphocytes to mitogen stimulation, suggesting a ‘combined immunodeficiency’. A recent study has identified the likely molecular defect in this condition by whole genome sequencing of an affected and control dog. The affected dog had a mutation in the *MKLN1* gene, which encodes muskelin 1, an intracellular protein with functions in cell adhesion, morphology, spreading and intracellular transport.

Deficiency of IgG has been documented in weimaraners, cavalier King Charles spaniels (CKCS) and as part of a more complex immunodeficiency in rottweilers and miniature dachshunds. Young related weimaraner dogs are reported with multisystemic inflammatory and infectious disease from the age of approximately 15 weeks. These dogs most consistently have subnormal concentration of serum IgG (sometimes with low IgM and IgA) and neutrophil functional defects have also been reported. Vaccination is thought to act as a trigger factor in this complex disease syndrome. Young CKCS and miniature dachshunds with pneumonia caused by *Pneumocystis carinii* also have subnormal serum IgG (often with compensatory elevation in serum IgM), and lymphocyte function defects have also been documented in affected miniature dachshunds.

Dogs of the rottweiler breed have long been suggested to have an immunodeficiency syndrome, related to their susceptibility to parvoviral infection and poor vaccine responses. A recent study of

serological responses to rabies vaccination has confirmed that the rottweiler is a low-responder breed. A litter of rottweiler pups with subnormal serum immunoglobulin concentrations and abnormalities in lymphoid tissue has been described, and another dog of this breed has been reported with deficiency in granulocyte-colony stimulating factor.

Only a single complement deficiency has been described in the dog. A research colony of Brittany spaniels has been well documented in the literature and the underlying molecular defect is described.

In addition to possible neutrophil dysfunction in weimaraners, abnormal neutrophil function was once documented in doberman pinschers with respiratory disease. A number of studies have examined the syndrome of cyclic haematopoiesis in grey collie dogs, and these animals have proven useful as an experimental model for therapeutic approaches to immunodeficiency (see below). The genetic mutation in this disease is in the gene encoding the β subunit of the adaptor protein 3 complex of neutrophils; the end effect is in the intracellular movement of neutrophil elastase. Another well-defined immunodeficiency affecting neutrophils is the CLAD (canine leukocyte adhesion deficiency; LAD 1) mutation in Irish (red, or red and white) setters. The neutrophils of affected dogs fail to express the adhesion molecules CD11b and CD18, so despite massive blood neutrophilia these cells cannot egress into tissues to fight infection. A similar disease (LAD 3) is rarely reported in the dog and is caused by defective β integrin activation due to mutations in the *KINLIN-3* gene. A persistent neutropenia was first described in border collie dogs in New Zealand in which there was apparent failure of release of neutrophils from the bone marrow (myelokathexis). This disorder is now known as the 'trapped neutrophil syndrome' and is recognized to be widespread in the border collie breed worldwide. A molecular diagnostic test is used to detect a 4 base pair deletion in exon 19 of the canine *VPS13B* gene, which encodes the vacuolar protein sorting-associated protein. The Pelger-Huet anomaly, characterised by hyposegmentation of granulocyte nuclei, has been described in American foxhounds, cocker spaniels, Boston terriers and basenjis. The observation that miniature schnauzers appear susceptible to disseminated mycobacterial infection has led to the proposal that this may reflect an underlying immunodeficiency, although there is no substantive evidence for this hypothesis to date.

The best-defined canine lymphoid immunodeficiency is severe combined immunodeficiency (SCID) described in the basset hound and corgi. Affected dogs have been studied in research colonies as a model for the equivalent disease in humans. Canine SCID involves a mutation in the common γ-chain of receptor molecules for the cytokines IL-2, IL-4, IL-7, IL-15 and IL-19. The disease is X-linked in the Basset but not the Corgi. More recently, a non-X-linked form of SCID has been described in a colony of Jack Russell terriers. The genetic defect in these dogs relates to defective DNA-dependent protein kinase that is responsible for molecular recombination giving rise to functional T and B lymphocyte receptors. This form of SCID is similar to that that has long been recognised in Arabian horses. The Jack Russell SCID mutation has been bred into beagle dogs to establish an experimental breeding colony for investigative purposes. A fourth form of canine SCID is recognized in Frisian water dogs and is caused by a mutation in the *RAG1* gene (also involved in lymphocyte receptor formation).

Mexican hairless dogs have been described as having subnormal immunoglobulin concentration, impaired delayed hypersensitivity response and depletion of lymphoid tissue. The association of hairlessness and immunodeficiency is well-recognised in inbred laboratory strains of 'nude' athymic rats and mice.

FELINE IMMUNODEFICIENCY

Although secondary immunodeficiency in this species is common (due to retroviral infection), primary congenital defects of the immune system are rarely reported. Thymic hypoplasia and associated hypotrichosis was identified in one litter of Birman kittens and more recent studies have identified the causative mutation in the *FOXN1* (forkhead box N1) gene. Sporadic thymic hypoplasia (not associated with parvovirus infection) is documented.

Feline leucocyte adhesion deficiency due to lack of expression of CD18 is now also identified, with the causative mutation in the *IGTB2* (integrin β2) gene of the single case recorded.

The Persian cat is one of a number of animals described as affected by the Chediak-Higashi syndrome, characterized by granulocyte dysfunction and altered pigmentation. Recent reports of disseminated mycobacterial infection in Abyssinian cats have proposed an underlying immunodeficiency that might explain this presentation.

FURTHER READING

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