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AUTOIMMUNE DISEASES (PARTS 1 AND 2)

Emeritus Professor Michael J. Day

Bristol Veterinary School, University of Bristol, UK
School of Veterinary and Life Sciences, Murdoch University, Western Australia

REGULATION OF THE IMMUNE SYSTEM

An understanding of autoimmunity requires knowledge of advances in understanding the mechanics of the basic immune response. It is now known that the two fundamental types of adaptive immune response (i.e. humoral or antibody-mediated immunity, and cytotoxic or cell-mediated immunity [CMI]) are initiated by the actions of specific populations of T helper cells (Th). These Th cells all carry the CD4 surface molecule and can only be discriminated by the panel of soluble signalling proteins (cytokines) that they produce after they are activated by their T-cell receptor recognizing an antigenic fragment (peptide) shown to them (presented) by an 'antigen-presenting cell' (APC; i.e. a dendritic cell or macrophage).

Accordingly, Th1 cells produce the cytokine interferon (IFN)-γ which provides an activation signal for CD8-expressing cytotoxic T cells, cytotoxic natural killer (NK) cells and phagocytic macrophages; allowing these cells to undertake cytotoxic destruction of a target cell (e.g. an infected cell, a cancer cell). In contrast, Th2 cells produce the cytokines interleukin (IL)-4, -5, -9 and -13, which provide activation signals for B lymphocytes, allowing them to transform into plasma cells when activated by exposure to antigen (via their B-cell receptor, which is a surface membrane antibody). Plasma cells then secrete antigen-specific antibody into the lymphatic fluid, blood and (if necessary) tissue spaces.

Th1 cells (and the related Th17 cells, which produce the cytokine IL-17 and stimulate cellular [granulocytic] immune responses in some infections and tissue-destructive autoimmune diseases) and Th2 cells share a common precursor, the Th0 cell. The nature of the antigen that is presented to the Th0 cell, the type of APC, the tissue environment in which the antigen is encountered, all determine whether the Th0 cell becomes a Th1, Th2 or Th17 cell and in turn, determines the nature of the immune response that is made to the antigen.

Finally, a separate population of $CD4^+$ T cells, the T regulatory cells (Tregs), is responsible for shutting off the adaptive immune response when it is no longer required (i.e. once antigen is destroyed). Treg cells produce a key cytokine, IL-10, which enables then to switch off (suppress or down-regulate) the 'effector' T and B cells, which participate in the active immune response. Treg cells are activated during the immune response to antigen ('induced Tregs'), but another important population of these cells ('natural Tregs') is always present in the body and works to prevent potentially autoreactive cells inducing autoimmune disease. Lack of number or function of natural Tregs is now known to underlie autoimmune diseases in man and animals.

AUTOIMMUNE DISEASES

The classical hallmarks of an autoimmune disease are:

- Compatible clinical signs (organ-specific or multisystemic)
- Absence of underlying disease (i.e. a primary idiopathic process)
- The presence of causative autoantibody or autoreactive lymphocytes
- Response to immunosuppressive therapy

Immunologically, autoimmunity is a state of failure of 'self-tolerance' to autoantigens, where the immune system inappropriately responds to self-tissue antigens leading to tissue pathology and associated clinical signs.

Human autoimmune diseases are relatively common (affecting around 10% of the population) and account for a major component of the human healthcare budget.

There is a wide spectrum of autoimmune disorders affecting both man and companion animals. The major small animal autoimmune disorders are:

- The immune-mediated blood dyscrasias (immune-mediated haemolytic anaemia [IMHA], thrombocytopenia [IMTP] and neutropenia [IMNP])
- Immune-mediated endocrinopathies (canine diabetes mellitus [probable], hypothyroidism and hypoadrenocorticism)
- Myasthenia gravis
- Exocrine pancreatic insufficiency
- Immune-mediated polyarthridities
- Pemphigus foliaceus and pemphigus vulgaris
- The spectrum of disorders involving an autoimmune response to epidermal basement membrane antigens (bullous pemphigoid variants)
- The spectrum of cutaneous lupus erythematosus disorders
- The erythema multiformae – toxic epidermal necrolysis spectrum
- Keratoconjunctivitis sicca and Sjogren's-like syndrome
- Chronic superficial keratoconjunctivitis
- Uveodermatitis
- Systemic lupus erythematosus

THE IMMUNOLOGY OF AUTOIMMUNE DISEASE

All clinically normal individuals harbour self-reactive T and B lymphocytes in their 'immunological repertoire'. These cells are programmed to express a receptor capable of recognizing epitopes from self-antigens. Normal individuals also often have low titres of 'physiological autoantibodies' that are generated to help remove dead cells arising from normal cellular turnover in the body. Autoreactive T and B cells must be controlled in order to prevent the onset of autoimmune disease. For autoreactive T cells a number of mechanisms are proposed. Many such T cells are deleted from the repertoire during the process of negative selection during intrathymic T-cell development. However, intrathymic deletion is not a 'failsafe' as such autoreactive T cells must 'escape' into the peripheral immune system in order to mediate autoimmune disease. These 'peripheral' autoreactive T cells can be controlled through a variety of mechanisms:

- Immunological ignorance (failure to recognize self-antigen)
- Peripheral deletion (death following recognition of self-antigen)
- Anergy (paralysis following recognition of self-antigen)
- Active control by regulatory T cells.

For autoreactive B cells, control may also come through a form of developmental selection, but in the main relies on the fact that the majority of B lymphocytes require 'help' from T cells to become activated. Therefore, controlling the activity of self-reactive T cells by default prevents activation of autoreactive B cells.

Immunologically, it is now recognized that patients with autoimmune disease have insufficient number or function of Tregs, permitting inappropriate activation of self-reactive T and B effector lymphocytes.

Research has been undertaken to investigate the autoantigenic targets of canine autoimmune responses. The molecular specificity of autoimmune reactions against erythrocytes, platelets, islet β cells, the neuromuscular junction, the epidermal desmosomes and the hemidesmosomes of the epidermal basement membrane and antinuclear antibodies has been reported. Such knowledge is important to progress to development of targeted immunotherapy for autoimmune diseases.

The nature of the tissue immune responses in autoimmune diseases is also being explored. One powerful tool in this respect has been the ability to undertake genomic screening of affected versus normal tissue to determine which genes are up- or downregulated within affected tissue. The involvement of these genes can then be confirmed by the use of specific quantitative real-time PCR reactions or immunohistochemical screening of tissues to evaluate protein expression and cellular distribution.

THE MULTIFACTORIAL NATURE OF AUTOIMMUNITY

Autoimmune disorders are multifactorial in nature, which is to say that the optimum combination of background predisposing and triggering factors must be in place in any one individual in order for an autoimmune reaction to occur and clinical manifestations of autoimmune disease to be recognized. The factors predisposing to autoimmunity include:

- Lifestyle, including diet and stress
- Genetic background
- Age
- Gender

The factors triggering autoimmunity include:

- Infection
- Neoplasia
- Exposure to certain drugs or vaccines
- Non-infectious tissue inflammatory disease
- Other environmental factors (e.g. UV light)

GENETIC BASIS FOR AUTOIMMUNE DISEASE

Autoimmune diseases have distinct predispositions in particular races of man, and are clearly inherited through families. However, studies of monozygotic twins show that other factors must impact on genetic background in order for autoimmunity to become expressed clinically. A similar situation exists for breeds of dog – but in feline medicine such associations are rarely documented. Examples of familial canine autoimmunity are also documented. One factor underlying this phenomenon is likely to be the degree of inbreeding which over the past two centuries has led to the development of many of the pure breeds of dog that we recognize today. Recent studies using microsatellite makers and investigation of specific gene polymorphisms have demonstrated the extent of inbreeding amongst some pedigrees. Such genetic influence might also underlie the apparent geographical prevalence of some immune-mediated diseases, for example canine autoimmunity appears to arise more commonly in Australia than in the UK which may reflect a 'founder effect' in some breeds.

Despite these observations, relatively little is understood of the precise genetic basis for autoimmune disease. Some gene clusters do have strong associations with immune-mediated disease, either because the protein products of the genes are integrally involved in the aberrant immune-response, or because the genes are 'markers' of poorly defined causative 'disease genes'. The best example of this are the associations between the genes of the Major Histocompatibility Complex (MHC) and autoimmunity. The class I, II and III genes of this complex are integral to immune function and are often termed ‘immune response genes’. Moreover, these are the most polymorphic genes within the genome with several hundred alleles now documented at some human class I loci. The products of class I and class II genes are involved in the presentation of antigenic peptides by antigen presenting cells (APC) for the activation of T lymphocytes. It is likely that particular allotypic variants of these MHC molecules might be most effective at presentation of self-peptides for triggering of auto-reactive T cell populations.

In consequence of this knowledge, the science of MHC-disease association has been widely developed in human medicine where it is clear that the inheritance of particular combinations (haplotypes) of MHC alleles either predisposes to, or protects from, development of autoimmunity. Similar studies have been conducted in the dog. In the late 1970s and early 1980s, using relatively crude serological and cellular methodology, associations were shown between canine autoimmune disease and MHC gene type. Over the past 20 years there has been a resurgence of interest in this field with the application of sophisticated molecular technology for determining the MHC genotype of an individual dog.

MHC-disease associations have now been clearly documented for canine immune-mediated haemolytic anaemia, lymphocytic thyroiditis, Addison’s disease, diabetes mellitus, sebaceous adenitis, rheumatoid arthritis (RA) and others. Similar associations have been shown to underlie

immunological susceptibility or resistance to infection by *Leishmania* and the susceptibility of German shepherd dogs to anal furunculosis. Both protective and susceptibility haplotypes for some of these diseases have been documented. Of interest is the investigation into RA where it has been shown that there is structural similarity between the MHC class II molecule encoded by the gene most highly linked to the disease phenotype in both man and dog. This in turn suggests that a common peptide antigen might be involved in triggering the disease in both species. In the case of diabetes mellitus and hypothyroidism, a common susceptibility haplotype suggests linkage in the mechanisms underlying autoimmune endocrinopathy. Additionally, single nucleotide polymorphisms (SNPs) in genes encoding the cytokines IL-4 and IL-10 have been associated with canine diabetes mellitus, but no association was demonstrated between TNF-α SNPs and anal furunculosis in the German shepherd.

GWAS involving microchip scanning of SNPs across the canine genome are now proving informative. One published study has defined genomic associations with the SLE-like autoimmune disease of the Nova Scotia duck tolling retriever. In canine IMHA there are genes linked to the disease in cocker and springer spaniels both at the family and population levels.

ROLE OF GENDER IN AUTOIMMUNITY

In man (but not dogs) there is a strong hormonal influence on the development of autoimmune disease with a marked female predisposition. Experimental studies in rodents also show the importance of hormonal balance. It is difficult to study the hormonal influence in canine autoimmune disease as many animals are neutered. One large epidemiological analysis of causes of death in some 40,000 dogs in a US multi-university referral database has shown an association between neutering and death from immune-mediated disease and cancer.

ROLE OF AGE IN AUTOIMMUNITY

There is a higher prevalence of autoimmunity in middle aged to older people and dogs. One of the proposed mechanisms by which increasing age is related to the development of autoimmune disease is that of 'immunosenescence'. The ageing immune system of dogs and cats displays similar changes to that occurring in geriatric humans. Ageing individuals have no impairment in the ability to maintain serum antibody, but there are imbalances in the lymphoid subsets within circulating blood, specifically reduction in $CD4^+$ T cells and B cells with associated elevation in $CD8^+$ T cells. It is considered that the reduced $CD4^+$ T cell population might include those regulatory T cells designed to keep potentially autoreactive lymphocytes in-check.

ENVIRONMENTAL INFLUENCES

The role of environmental factors is now recognised to be of major importance in the expression of autoimmune disease. A major area of research in human clinical immunology has been the 'hygiene hypothesis' which has been proposed to account for the increasing incidence of both allergic and autoimmune disease in western society. Understanding of this area of immunology requires knowledge of the current model of T helper (Th) cell subsets as described earlier.

It is known that the fetal immune system *in utero* is dominated by type 2 (Th2) immunity. This arises as a consequence of re-direction of the maternal immune response away from Th1 responses that may potentially be involved in fetal loss. All new-born human infants, and also new-born puppies, are born with an imbalanced immune system with dominance of Th2 immunity and lack of sufficient natural suppressor cells (Treg). In neonatal life and infancy the immune system is redirected and 'fine-tuned' by exposure to infectious agents which have a net effect of expanding the population of protective Treg cells. The modern emphasis on a sanitised lifestyle, together with administration of neonatal vaccines in alum (an adjuvant known to preferentially induce type 2 immune responses) contributes to a failure to develop adequate regulatory T cells and increased susceptibility to allergic and autoimmune disease. This hypothesis is supported by numerous epidemiological and immunological studies which show protective effects (from developing immune-mediated disease) if children are brought up in a rural *versus* urban environment, have exposure to pets in the home, attend nursery or are part of large families or receive BCG vaccination. Exposure of the pregnant mother to such influences may also be able to direct programming of the developing fetal immune system towards resistance to allergic disease.

A recent study has compared the association between urban and rural environment and canine immune-mediated haematological, joint and CNS disease. Although the odds ratio was no different between these two backgrounds, this may simply reflect exposure to different trigger factors in the different environments.

Another lifestyle factor contributing to the development of immune-mediated diseases is obesity. Adipose tissue is regarded as an immunological tissue and obese individuals have increased expression of molecules such as leptin and proinflammatory cytokines such as IL-6 and TNF-α. This background leads to an increased level of chronic inflammation and Th1 activity in obese individuals, which in turn may trigger autoimmunity. Altered function of lymphocytes has been demonstrated in an experimental study of obese beagle dogs.

A season influence on the occurrence of IMHA has been reported in the USA (Spring – Summer), Europe and Australia (Summer – Autumn). Difficult to interpret, this might simply reflect the greater exposure to other risk factors during certain seasons (e.g. exposure to arthropod-borne infections, increased likelihood of kennelling during vacation). Exposure to UV light has been suggested to impact on some immune-mediated skin diseases, perhaps by direct alteration of autoantigens creating novel target molecules for immune reactivity.

Infection is also now known to play a key role in the development of autoimmune disease, and in human medicine it has been proposed that autoimmunity might be triggered by previous or contemporary exposure to microbes. Viruses are widely implicated as the trigger of many human 'autoimmune' diseases. In companion animal medicine we also now appreciate that infectious agents might underlie diseases that were previously considered 'idiopathic autoimmune' in nature. The largest group of such organisms are the arthropod-transmitted agents which appear to have a propensity for interacting with the host immune response to trigger secondary immune-mediated phenomena. The classical example of such an effect occurs in canine leishmaniosis. The clinical manifestations of symptomatic visceral leishmaniosis largely related to immune-mediated phenomena such as autoantibody induction (IMHA and IMTP) and immune complex deposition (glomerulonephritis, polyarthritis). In canine rheumatoid arthritis a potential trigger is CDV infection with deposition of CDV immune complexes in the synovium and in other forms of immune-mediated polyarthritis a bacterial trigger is implied. In some canine immune-mediated diseases, PCR of lesional tissue has been employed to attempt to identify triggering infectious agents, but this is not always enlightening.

Extending from the hygiene hypothesis discussed above has been the recognition that the balance of microorganisms in the intestinal microbiome is crucial for the optimum number and function of Tregs in the immune system. It is now known that imbalance (dysbiosis) can lead to reduced Treg activity and inappropriate activation of effector Th1 and Th17 cells in human autoimmune diseases of the joints, CNS and pancreas. The obvious corollary to these studies has been attempts to restore immune homeostasis (and elevate Treg activity) through the use of pre- and probiotics. Experimentally, it has been shown that a cocktail of specific clostridial species can induce Tregs and prevent T-cell-mediated colitis in mice. It has also been suggested that there are gender influences on this action of the intestinal microbiome. Female NOD mice develop type 1 diabetes with a much higher prevalence than male mice of this strain. Giving male (but not female) caecal content to female NOD mice protects them from development of the disease.

There also appears to be a correlation between intestinal parasitism and protection from immune-mediated diseases, thought to relate to the ability of nematodes to direct the development of Treg populations. This relationship explains the very low incidence of immune-mediated disease in third world populations, and the profound clinical benefit that may be noted in patients with allergic disease or inflammatory enteropathy (Crohn's disease) when deliberately infected with *Trichuris suis* eggs (orally) or hookworm larvae (percutaneously). The specific 'excretory–secretory' antigens within parasites that are responsible for these effects are now being investigated. Pilot studies have also evaluated the effect of 'parasite immunotherapy' on canine allergic disease.

FURTHER READING

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