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IMMUNOLOGY AND VACCINOLOGY

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Michael Day qualified as a veterinary surgeon from Murdoch University (Western Australia) in 1982. After a period in small animal practice he returned to Murdoch to complete a Residency in Microbiology and Immunology, and a PhD involving collaborative research with the Royal Perth Hospital. Michael held postdoctoral positions in experimental immunology at the Universities of Bristol and Oxford and in 1990 returned to Bristol where he is currently Professor of Veterinary Pathology. His research interests cover experimental models of autoimmunity and a range of companion animal immune-mediated and infectious diseases. Michael has published widely in the field of immunopathology, is author of the textbooks *Clinical Immunology of the Dog and Cat* (in second edition) and *Veterinary Immunology: Principles and Practice* (in second edition). He is co-editor of the *BSAVA Manual of Canine and Feline Haematology and Transfusion Medicine* (in second edition) and the texts *Arthropod-Borne Infectious Diseases of the Dog and Cat* (second edition in preparation) and *Canine and Feline Gastroenterology*. He is a diplomate of the European College of Veterinary Pathology, and holds fellowship of the Australian Society for Microbiology, the Royal College of Pathologists and the Royal College of Veterinary Surgeons. Michael is Editor-in-Chief of the *Journal of Comparative Pathology*. He is a Past President of the BSAVA. Michael is also chairman of the WSAVA Vaccination Guidelines Group and the WSAVA One Health Committee. He is a member of the Petplan Charitable Trust Scientific Committee, Vice President of the WSAVA Foundation, a member of the Board of the WSAVA AFSCAN Project and a Trustee for the Mission Rabies Project. Michael is co-founder of a university spin-out company KWS Biotest Ltd where he is Director of Pathology. He has been the recipient of the BSAVA Amoroso Award for outstanding contribution to small animal studies (1999), the BSAVA Petsavers Award (2000, 2006 & 2007), the RCVS Trust's G. Norman Hall Medal for outstanding research into animal diseases (2003) and the Petplan Charitable Trust Scientific Award (2009).

HOW DOES THE IMMUNE SYSTEM WORK – AND WHY DO I NEED TO KNOW?

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Introduction

This lecture will use the model of the cutaneous immune system to explain and discuss the way in which the immune system functions to protect the body from challenge by pathogenic organisms. The skin is the largest organ of the body and its vast surface area means that it is continually bombarded with potential pathogens, allergens and inert particles. The skin is protected by both innate and acquired immune systems which function in similar fashion to those providing immune defence of mucosal surfaces. The skin immune system (SIS) works in conjunction with the skin-associated lymphoid tissue (SALT) to provide immunological protection at this site. As for other 'front line' areas of the body (i.e. the mucosal surfaces), the skin immune system is likely to continually sample a range of antigens (heteroantigenic pathogens or allergens, self antigens) and must make fundamental decisions about whether to respond to the antigen by fully activating the immune system (to produce a protective response in times of 'danger') or ignoring the presence of the antigen (to produce immunological tolerance to self antigens or endogenous microflora). Occasionally, this decision making fails and the skin immune system will respond inappropriately to antigens – resulting in clinical allergic or autoimmune disease.

Innate Immunity

The innate immune system of the skin includes the fundamental barrier to antigenic exposure provided by the tough, dry, keratinized environment of the epidermis. Coupled with the presence of endogenous microflora and the various inhibitory and enzymatic constituents of glandular secretion (e.g. sebaceous and keratinocyte-derived lipids), the surface of the skin is generally considered relatively inhospitable to foreign antigen. The nature of the cutaneous barrier depends on the anatomical location – as there are areas which provide a more conducive environment to pathogen survival (e.g. sparsely haired, relatively humid ventromedial surfaces, the external ear and mucocutaneous junctions).

In addition to the simple barrier, the innate immune system comprises a range of cells and molecules that are continually present at the cutaneous surface and provide local 'immune surveillance' and an instant, 'rapid response' to inappropriate allergen exposure. The molecules will include immunoglobulin and components of the alternate pathway of complement which may be constituents of the glandular secretions that bathe the cutaneous surface. Techniques such as skin-washing or the formation of suction blisters have shown the presence of immunoglobulin (predominantly IgA) and plasma cells (particularly those expressing IgA or IgG) are normally found in association with apocrine glandular elements of the skin. The nature of immunoglobulin secreted onto the normal cutaneous surface is likely to be similar to that found on mucosae, and comprise a 'polyreactive' immunoglobulin capable of binding numerous different antigenic epitopes with relatively limited affinity. Small antimicrobial cationic peptides (β-defensins and cathelicidins) are also recognized as an important component of cutaneous innate immunity. These are derived from keratinocytes and immune cells and may directly mediate microbial killing in addition to acting as leukocyte chemoattractants and immunomodulators. Keratinocytes may also constitutively secrete (or be induced to secrete) a range of cytokines and chemokines (see later) including interleukin (IL)-1, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-18, IL-20, tumour necrosis factor (TNF)-α and interferon (IFN)-α, IFN-β and IFN-γ.

The normal skin also contains a distinct population of leukocytes which may be found within the epidermis or the superficial dermis, particularly adjacent to small capillaries. This fundamental pattern is again mirrored in the mucosal surfaces of the gut and respiratory tract. Within the epidermis may be found the population of T lymphocytes that express the γδ T cell receptor (TCR). These intraepithelial lymphocytes are also found within the epithelial barrier of the mucosae and are regarded as a more primitive population consistent with an innate immune role. In the intestinal tract, γδ T cells have been shown to have exquisite reactivity to a range of bacterial antigens (derived from potential pathogens) and are thought to be important in providing 'first-line' defence to such agents. γδ T cells are generally regarded as being derived from the thymus but in some species there is a suggestion for local mucosal generation. There are also species

differences in the distribution of this population – in cattle, γδ T cells comprise a significant population within the circulating blood, and in dogs there is a proportionally high number of these cells that reside within the spleen. Although γδ T cells are identified in the epidermis, and may increase in number in immune responses (e.g. to allergens), their precise role in the skin immune system is poorly defined. There may also be important species differences: while intraepidermal T cells are found in the skin of rodents, ruminants and people, they have not been identified in cats and horses.

A range of leukocytes is found within the superficial dermis, including mast cells, macrophages and occasional granulocytes. Mast cells are found at a similar subepithelial (or rarely intraepithelial) location in the gut and respiratory tract and are often immediately adjacent to small capillaries. These cells may also be coated with FcεRI-bound IgE and thus function as a means of rapidly inducing regional vasodilation and recruitment of inflammatory and immune cells in times of antigenic exposure. Macrophages are an important constituent of the innate immune system that are able to non-specifically phagocytose and remove any particulate material that may penetrate the barrier defences of the skin. At mucosal surfaces, natural killer (NK) cells are considered an important constituent of the innate immune system but resident NK cells have been poorly described in the skin. These cytotoxic lymphoid cells have the capacity to destroy foreign or modified target tissue cells through recognition of surface antigen directly via their NK cell receptor, or alternatively via recognition of antibody bound to the target cell surface via their Fc receptor (antibody-dependent cell-mediated cytotoxicity; ADCC). The NK cell can only recognize and destroy a target cell if it fails to express class I molecules of the major histocompatibility complex (MHC), as the NK cell also expresses an inhibitory receptor that binds to class I on normal tissue cells and prevents cytotoxicity from occurring.

The most important part of the innate immune system is however, the dendritic cell. Until relatively recently, immunologists rather dismissed the innate immune system as uninteresting and simple – and preferred to study the more complex interactions of adaptive immunity. However, there is now a recognition that the dendritic cell is the key link between innate and adaptive immunity – and moreover, that the dendritic cell is responsible for directing the nature of the adaptive immune response that is generated in response to antigen. The two key types of dendritic cell within the SIS are the epidermal Langerhans cells and the dermal dendritic cells. The Langerhans cell is ideally located within

the epidermis where its dendritic processes may extend between keratinocytes to optimally make contact with antigen penetrating the epidermal barrier. The dermal dendritic cells will be exposed to antigen that percolates through the epidermis, or perhaps is directly injected (e.g. arthropod saliva) into the dermis thus bypassing the epidermal barrier. These dendritic cells may be identified by their constitutive expression of class II MHC molecules and via other lineage-specific molecules such as CD1. Dendritic cell number may expand during inflammatory and immune responses and they may upregulate expression of these key molecules. Epidermal Langerhans cells may also express IgE bound to the low affinity IgE receptor CD23, and this expression may be important in perpetuating hypersensitivity responses to allergens that penetrate the epidermis.

The recent focus on dendritic cells however has arisen through the discovery of a series of molecules expressed by these cells (and also by keratinocytes) that are known as 'pattern recognition receptors' (PRRs; or alternatively 'Toll-like receptors'). These PRRs are designed to interact with a select number of highly conserved molecules or constituents (e.g. lipopolysaccharide, DNA) of potential pathogens – known collectively as 'pathogen-associated molecular patterns' (PAMPs) or (to take into consideration the microbiome) 'microorganism-associated molecular patterns' (MAMPs). PRRs may also recognize non-microbial molecules that are generated during damage to normal tissue ('damage-associated molecular patterns' [DAMPs]). The PRRs expressed by dendritic cells have been likened to the bar-code readers found in supermarkets – the dendritic cells in effect are 'scanning' the surface of foreign antigens looking for molecules that they might recognize and provide the identity of the antigen. Other PRRs are located within the cytoplasm of the dendritic cell (e.g. the nucleotide-binding oligomerization domain [NOD]-like receptors) and interact with internalized antigen. The interaction between a PAMP and dendritic cell PRR sets up a complex pathway of signal translocation from cell membrane to nucleus of the dendritic cell which instructs the dendritic cell to become activated, to express particular co-stimulatory molecules on its surface, and to secrete particular cytokines. It is the nature of these co-stimulatory molecules and cytokines that subsequently directs the outcome of the adaptive immune response by preferentially activating Th1, Th2, Th17 or regulatory T cells. It is also likely that similar interactions may direct a tolerance (rather than activation) response. In the context of the SIS, it would seem that the 'default' response to percutaneous absorption or penetration of antigen is for Langerhans cells and dermal

dendritic cells to stimulate Th2 cells and type 2 immunity. This may in part underlie the propensity for the SIS to generate allergic responses. By contrast, there are clearly antigens that penetrate the cutaneous defences that are capable of inducing a strong Th1/Th17 protective immune response (e.g. *Leishmania*, *Mycobacterium*, dermatophytes). The NOD-like PRRs are an important component of an intracellular complex of molecules known as the 'inflammasome'. Formation of the inflammasome promotes the activation of caspase-1 which cleaves pro-IL-1β into the active and secreted form of IL-1β - a key proinflammatory cytokine.

Adaptive Immunity

The adaptive immune response follows the engagement of innate immunity and antigen encounter with cutaneous dendritic cells. Although much more specific and potent, the adaptive immune response will generally take 7-10 days (in the case of primary exposure to antigen) to become sufficiently activated to contribute to the local cutaneous immune defence. A memory adaptive immune response may be generated in significantly less time.

The adaptive immune response is not engendered in the microenvironment of the skin, but occurs in the regional draining lymphoid tissue (SALT). The reason for this is to maximize the chance of contact between foreign antigen and that subset of lymphocytes that bear pre-programmed receptor molecules capable of interacting with epitopes from that antigen. Lymphocytes continually recirculate between blood, lymph and lymphoid tissue – effectively patrolling the body on the lookout for their cognate antigen (immune surveillance) that may enter the body at a variety of possible points. Those lymphocytes that are pre-programmed to recognize a specific antigen must be able to access those points of the body where contact with that antigen is most likely.

Antigen must therefore be translocated from the skin to the regional lymph node in order to maximise the chance of contact with those T and B lymphocytes that bear relevant receptors. The route from skin to lymph node is via the afferent lymphatic vessel that drains lymphatic fluid and cells into the subcapsular sinus of the lymph node and thence into the nodal tissue through to the medullary sinus. Whilst it is possible for antigenic particles to travel directly in lymphatic fluid, it is more efficient for them to be carried by mobile macrophages or dendritic cells. The dendritic cells are likely key to this event, and whilst travelling within the afferent lymph they undergo a morphological change from a

'dendritic' to 'veiled' phenotype. On arrival in the lymph node, the antigen-exposed dendritic cells localize to the paracortex of the node where they are most likely to encounter T lymphocytes. This interaction between dendritic cell and potential cognate T cells has now been studied by real-time imaging. It is estimated that up to 500 different T cells every hour will come up to a dendritic cell to determine if it bears antigen that may be recognized by the TCR of the T cell. The migration of cutaneous dendritic cells has been studied in a murine model in which Langerhans cells were genetically engineered to express green fluorescent protein. After red fluorescent dye (TRITC) was applied to the skin and percutaneously absorbed, the Langerhans cells (yellow) and dermal dendritic cells (red) were shown to localize to distinct areas of the draining lymph node paracortex.

The dendritic cell not only internalizes the foreign antigen, but must 'process' and 'present' it in a form that the T cell can recognize. There are two intracytoplasmic processing pathways used by dendritic cells. The most commonly employed is that pathway that deals with 'exogenous' antigens derived from outside of the dendritic cells (the majority of antigens encountered by the skin). Here, the antigens are internalized into a cytoplasmic digestion chamber where they are broken down by enzymatic degradation into small peptide fragments of the original antigen (in the order of 20-25 amino acids in length). Lining the inner surface of the digestion chamber are class II molecules of the MHC. The enzymes within the chamber cleave the MHC-associated 'invariant chain' and permit the peptide fragments to associate with the variable regions (antigen binding) of the class II molecules. Once this is achieved, the chamber relocates to the margin of the cell and merges with the cell membrane such that the peptide-loaded MHC molecules are now exposed on the surface of the cell (antigen presentation). The alternative 'endogenous' pathway deals with antigens that are generated within an antigen presenting cell (APC) such as virally-derived molecules, self antigens or tumour antigens. These antigenic structures undergo proteolytic degradation within a distinct cytoplasmic compartment known as the 'proteasome'. The peptide fragments generated (10-15 amino acids) are relocated to the endoplasmic reticulum via a 'transporter' molecule where they are loaded into the antigen-binding (variable) region of class I MHC molecules. These peptide-loaded MHC molecules also become expressed on the APC surface – a process that likely involves a route through the Golgi apparatus of the cell, but is poorly understood. It is possible for either type of

antigen to 'cross-over' into the other processing pathway so both MHC I and II expression of peptide may occur in some circumstances.

The dendritic cell within lymph node paracortex is therefore fully activated and expressing processed antigenic peptide on MHC class I or II molecules, together with a range of other surface co-stimulatory molecules. Additionally, the dendritic APC will be secreting cytokine. The nature of the co-stimulation and cytokine will have been determined by the type of antigen and in turn will determine the type of adaptive immune response that subsequently ensues.

In the case of a naïve immune response, the type of T cell that will encounter the presented antigen will classically be a relatively undifferentiated Th0 cell. These are $CD4^+$ T cells that bear an $\alpha\beta$TCR specific for antigen but are not yet functionally committed to a Th1, Th2, Th17 or Treg immune response. The encounter with APC will direct the Th0 cell to differentiate to generate a dominant Th1, Th2 or Th17 effector response. The APC-T cell interaction involves recognition of antigenic peptide-MHC complex by the TCR, recognition of MHC by the T cell CD4 molecule, a series of other surface molecular interactions (e.g. T cell CD28 with APC CD80/86), and the delivery of APC-derived cytokine to specific cytokine receptors expressed on the T cell membrane. These stimulatory events are often referred to as 'signal 1', 'signal 2' and 'signal 3' respectively. For example, a dendritic cell that has processed and presented antigen derived from *Mycobacteria* or *Leishmania* will signal the Th0 cell with cytokines such as IL-12 and IL-18 inducing that cell to react through the stat 4 pathway (stat = signal transduction and activation of T cells) to drive that Th0 cell into becoming an IFNγ–secreting Th1 cell that mediates the appropriate protective immune response to the organism. By contrast, antigen derived from house dust mite or flea saliva may more readily presented by an APC that signals the Th0 cell through IL-6, thus activating the stat 6 pathway and leading to differentiation towards a Th2 cell secreting IL-4, IL-5, IL-6, IL-9 and IL-13. Th17 cells are characterized by production of the cytokines IL-17A, IL-17F and IL-22 and are involved in pathogen (particularly bacterial and fungal) clearance and autoimmune and allergic reactions. In particular, Th17 cells have a role in cutaneous *Staphylococcus* infection through IL-17-mediated neutrophil recruitment. It is also likely that the APC-T cell encounter is responsible for the induction of regulatory T cells responsible for suppression, rather than activation, of immune responses. Treg induction might arise when antigen is presented by a less mature dendritic cell that may signal in a

distinct fashion to those APC that induce effector populations. It is now recognized that this T cell lineage differentiation is not absolute and that it is possible for one type of mature effector to transform into another (e.g. Th1 to Treg) at a particular stage of an immune response. This is referred to as T-cell 'plasticity'.

Stimulation of the antigen-specific T cell in this fashion will lead to clonal expansion of this population to amplify the number of antigen-relevant T cells. Some antigen-specific T cells migrate into the margins of primary B cell follicles within the lymph node cortex where they provide help for antigen-specific B cells that independently recognize large, conformational determinants of antigen translocated to the lymph node. T cell help for the majority of (T-dependent) antigens involves physical contact between the T and B cell in addition to the release of co-stimulatory cytokines from the Th cell. B cells recognize antigen via the B cell receptor (BCR) which comprises of surface membrane immunoglobulin (of the IgM and IgD classes in naive B cells). Antigen is internalized by the B cell and undergoes processing as described above for the endogenous pathway of classical APC – resulting in the expression of antigenic peptide-MHC class II on the surface of the B cell. The cognate interaction between T and B cells thus involves similar molecular interactions as described for T cells and the APC. In particular, TCR engagement via recognition of peptide-MHC (signal 1), the interaction between T and B cell surface molecules (e.g. T cell CD40 ligand and B cell CD40; signal 2), and the delivery of co-stimulatory cytokine from the T cell (e.g. IL-4, IL-5, IL-6, IL-13; signal 3). Activated B cells subsequently undergo clonal proliferation with the formation of a germinal centre within the secondary lymphoid follicle. Activated B cells undergo the 'immunoglobulin class switch' whereby dual expression of the immunoglobulin heavy chain μ and δ genes is replaced by single expression of a single heavy chain gene (usually γ, α or ε) resulting in expression of a single immunoglobulin class on the B cell membrane. The class-switch is directed by cytokine signalling which determines the nature of the humoral immune response made to the initiating antigen – and again the initial interaction with dendritic cells underpins this outcome. Within the lymphoid follicle, activated B cells again migrate to the margins of the follicle to re-encounter both antigen-specific T cells and follicular dendritic cells bearing processed antigen. This further encounter acts as a checkpoint for developing B cells – only those with BCRs capable of high affinity interaction with antigen are permitted to survive and continue to the effector or memory pathways of the immune response. The former, involves the terminal

differentiation of B cells to plasma cells that secrete immunoglobulin of the chosen class and specificity. At least some of this differentiation and antibody production will occur in the medullary cords of the draining lymph node.

The problem next facing the immune system is how to mobilize this cohort of antigen-specific T and B cells and direct them back to the cutaneous site of antigen encounter where they are required to generate the effector phase of the immune response to eliminate antigen (or potentially elicit an inappropriate allergic or autoimmune response). The antigen-specific lymphocytes leave the draining lymph node via the efferent lymphatic, which in turn drains into the major thoracic duct and thence into the blood circulation. The lymphocytes travel in the circulating blood until they reach the specific cutaneous site where they are required. This recognition is made possible by modifications of the vascular endothelium at the site of inflammation – the transformation to a larger, cuboidal endothelial lining cell (high endothelial venule; HEV) that causes local turbulence of blood flow thus enhancing the interaction of leukocytes with endothelium. Moreover, this transformed endothelium expresses a range of adhesion molecules ('vascular addressins') unique to this site (e.g. E-selectin, ICAM-1, VCAM-1) which selectively interact with ligands ('homing receptors') that are expressed by the antigen-specific lymphocytes (e.g. cutaneous lymphocyte antigen; CLA). In fact, recirculating naïve lymphocytes probably express an array of such homing receptors, but when stimulated by antigen in the context of a specific anatomical site are likely to down-regulate all but those homing receptors which will enable them to exit the circulation at the anatomical location where they are required.

Once the antigen-specific T and B cells have entered the dermal microenvironment, they then proceed to generate the fully fledged effector phase of the acquired immune response. The nature of this will be determined by the initiating antigen and the original encounter with APC. In a Th1 (type 1) dominated immune response (e.g. to *Leishmania*), the Th1 cells will secrete IFN-γ that acts on parasitized macrophages to enable them to kill intracellular amastigotes. The IFN-γ may further up-regulate adhesion molecule expression on local vascular endothelium and may induce activation of regional APC that can amplify and perpetuate the local immune response. IFN-γ in this context may also act on keratinocytes and dermal fibroblasts causing them to participate in the immune response by expressing MHC class II and antigen (acting as 'non-professional' antigen presenting cells). Keratinocytes are also capable of expressing a wide range of cytokines and chemokines that

provide further amplification of local immunity. In the case of a response to local viral infection (e.g. cowpox) or neoplasia (e.g. canine histiocytoma), NK cells and classical $CD8^+$ cytotoxic T cells may be recruited and activated to destroy target cells. In a type 1 immune response, only limited antibody production will occur (generally a restricted subclass of IgG), but this antibody may be important in mediating ADCC or opsonizing free antigen for macrophage phagocytosis. In a type 2 immune response (e.g. to aeroallergen or ectoparasites) the effector phase will be dominated by antibody production by locally differentiated plasma cells. The locally produced IgE or reagenic IgG antibody will occupy mast cell receptors, leading to antigen cross-linkage and mast cell degranulation which amplifies the local immune and inflammatory response. In both types of response, cells such as T lymphocytes, macrophages and epithelia will produce another class of soluble mediator – the chemokine. There are numerous chemokine families (defined structurally), the members of which are responsible for chemotactic recruitment of relevant leukocytes from the circulation into the site of inflammation. Like cytokines, chemokines act by binding to specific receptor molecules expressed by the recruited cells. Examples of chemokines include IL-8 which recruits neutrophils into tissue, the monocyte chemotactic proteins (MCP) which primarily recruit monocytes, and the eotaxins which recruit eosinophils. Not surprisingly, eotaxin expression is upregulated in the cutaneous type I hypersensitivity response. Chemokines are also involved in the initial interaction between circulating leukocytes and vascular endothelium – for example, CLA^+ skin homing T cells also express the chemokine receptor CCR4 which interacts with the chemokine CCL17 (TARC) found on the endothelial surface.

The entire purpose of triggering the chain of events described above is to generate an immune response to 'danger' that results in elimination of the inciting antigen (pathogen) and a return to normal tissue homeostasis. Of course, in some situations this does not occur and the persistence of the response leads to disease or death of the animal (e.g. failure to eliminate *Leishmania* leading to death of the susceptible animal, failure to down-regulate an allergic or autoimmune response, or failure to destroy a neoplasm). Although the down-regulation of an active immune-response might in part simply reflect destruction of antigen will lack of sufficient antigen to drive the response, it is now believed that suppression of the immune response is an active event predominantly mediated by regulatory T cells. Increasingly, evidence points to $CD4^+$ T cells as being dominant in this

capacity. Whilst much significance was once placed on the antagonistic effects of Th1 and Th2 cells in mediating regulation by counterbalancing the function of each other, it is now recognized that specific regulatory or suppressor populations of T cell are generated in any immune response, and that these act to mediate the terminal suppression of the response when it is no longer required. In fact, failure to generate such suppression results in uncontrolled activation of effector cells that underlies allergic or autoimmune disease.

In experimental animal systems, a variety of regulatory T cells are now recognized. In normal animals, those $CD4^+$ T cells that also express CD25 are regarded as 'natural suppressors' (Treg) and work to inhibit the action of effector cells by direct physical interaction with their targets. Natural suppressors are also defined by the expression of surface CTLA-4, some PRRs and GITR (glucocorticoid-induced TNF receptor-related gene) and by expression of the gene known as Foxp3. In experimental disease models, further populations of 'induced suppressor' cells are recognized. These cells (Tr1) inhibit effector function via release of the cytokine IL-10 and do not require physical interaction with their targets. At mucosal surfaces, a population of Th3 cells that selectively produces the cytokine TGFβ is also suppressive in function and may mediate the phenomenon of 'oral tolerance'.

The final act in generating an immune response is to ensure the development of immunological memory to the inciting antigen. Memory lymphocytes are very poorly understood and difficult to identify for experimental study. Memory T cells may express particular isoforms of the common leukocyte antigen (CD45) but markers of memory B cells are not described. Memory cells are more sensitive to antigen and able to react more efficiently and quickly to induce the potent memory immune response. Memory cells are regarded as being relatively long-lived lymphocytes, but the duration of this longevity is debated. It is suggested that memory cells are maintained in the body by periodic low-level division following encounter with residual antigen that may be retained in association with dendritic cells within lymphoid tissue.

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IMMUNOPATHOLOGICAL MECHANISMS

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Introduction

An individual who is repeatedly exposed to an antigen over time (sensitized) develops the capacity to mount an inappropriately excessive immune response (hypersensitivity reaction) on subsequent exposure to that antigen. The antigens (allergens) that drive such responses are often ubiquitous environmentally-derived substances to which only genetically predisposed individuals will react in this fashion. The hypersensitivity response will generally result in cellular and tissue changes and the clinical manifestations of allergic disease. However, the same immunological mechanisms are also utilized in certain autoimmune diseases and in the immune response to infectious agents – so these may best be considered as 'immunopathological mechanisms' rather than 'hypersensitivity mechanisms' *per se*.

The hypersensitivity mechanisms were first defined in 1963 by Gell and Coombs and their classification of Type I – IV reactions still underpins our understanding of clinical immunology. This presentation will review the fundamental basis for these reaction types.

Type I (Immediate) Hypersensitivity

As for all of these reactions – type I hypersensitivity occurs in two phases – an initial phase of immunological sensitization, followed by the subsequent antigen challenge and hypersensitivity reaction. In the first phase, antigen (allergen) is captured by peripheral dendritic cells (at mucosal or cutaneous surfaces) and translocated to regional draining lymphoid tissue where the antigen presenting cells (APC) drive a Th2-regulated expansion of B cells committed to production of allergen-specific IgE (and particular 'homocytotropic' IgG subclasses depending upon species). These immunoglobulins (Igs) are bound by FcεR1 on the surface of circulating basophils and tissue mast cells – and the individual is thus immunologically 'sensitized'. The cell-bound IgE may persist for many months and when there is re-exposure to allergen, antigenic epitopes are bound by the allergen-specific Ig resulting in 'cross-linking' of the molecules and perturbation of the cell membrane which

triggers a complex series of intracytoplasmic signalling events. These in turn result in degranulation of the mast cell or basophil with local release of the cocktail of potent proinflammatory and immunoregulatory molecules which are either pre-formed in the cell or synthesized *de novo* on activation. Mast cell mediators include: tryptase, chymase, carboxypeptidase, cathepsin G, histamine, heparin, kininogenase, serotonin, IL-3, IL-4, IL-5, IL-1, GM-CSF, TNF-α, leukotrienes B_4, C_4 and D_4, thromboxanes, prostaglandin D_2 and platelet-activating factor.

The clinical consequences of mast cell degranulation become evident following the effect of these mediators on vascular endothelium (resulting in tissue oedema and egress of inflammatory cells and proteins), bronchial smooth muscle (resulting in bronchoconstriction) and local neurological cells (resulting in cutaneous pruritus). These reactions occur within minutes of exposure to allergen (thus 'immediate' hypersensitivity), but it is now recognized that a 'late phase' to this response occurs at approximately 4 to 24 hours which is characterized by local tissue influx of eosinophils and macrophages. Such type I reactions may be anatomically localized (e.g. atopic dermatitis or asthma) or systemic (e.g. anaphylaxis). Numerous investigations have determined that the expression of type I hypersensitivity is multifactorial, and involves a combination of factors such as genetic background, environmental exposure to allergen, age, stress and exposure to infectious agents or parasites (the 'hygiene hypothesis'). These predisposing factors contribute to a failure of immunoregulation mediated by $CD4^+$ regulatory T cells which permits development of immunological sensitization.

Type II (Cytotoxic) Hypersensitivity

This form of hypersensitivity reaction also involves the production of IgG (less often IgM) antibody in response to antigen exposure. The target antigen is generally associated with the membrane of a cell which becomes the target of an antibody-mediated cytotoxic immune response. This cytotoxic destruction may be mediated by: (1) activation of complement following binding of antibody to target cell with formation of the terminal membrane attack complex and osmotic lysis of the target, (2) phagocytosis of the antibody opsonized target by a macrophage bearing Fc and C3b receptors, or (3) antibody-dependent cell-mediated cytotoxicity (ADCC) performed by a Natural Killer (NK) cell. The most common clinical example of type II hypersensitivity is a blood transfusion reaction where a recipient

animal with preformed circulating alloantibodies is transfused with incompatible donor blood. Binding of antibody to transfused erythrocytes may lead to a spectrum of acute anaphylactic (type I hypersensitivity) or chronic haemolytic (type II hypersensitivity) reactions. The same immunological events underlie diseases such as IMHA or IMTP.

The diseases involving anti-receptor antibody formation also fit into this category of immunopathological mechanism. These are best characterized by human Grave's disease (anti-TSH receptor antibodies leading to inappropriate over-activation of the thyroid) or myasthenia gravis in humans, dogs and cats (anti-AChR antibodies leading to blockade or down-regulation of neuromuscular AChR).

Type III (Immune Complex) Hypersensitivity

This form of hypersensitivity reaction again involves the formation of IgG antibodies and their combination with antigen to form immune complexes. Two distinct scenarios occur depending upon the relative proportions of antigen and antibody involved in the reaction. In 'antibody excess' the individual is sensitized to antigen such that large numbers of IgG molecules are generated. On subsequent exposure to antigen (generally via inhalation) there is rapid binding of the antigen by this IgG at the local site of exposure. These locally-formed immune complexes generate an intense localized inflammatory response known as an 'Arthus reaction'. The inflammation involves activation of complement, regional vasodilation, recruitment and activation of leukocytes with production of pro-inflammatory mediators. Such reactions are rare in veterinary medicine.

The alternative mechanism involves a situation of 'antigen excess' where there is exposure to high concentrations of antigen over the quantity of IgG present. This situation leads to the formation of smaller, soluble immune complexes within the circulation rather than localized within the tissue of antigen exposure. These complexes lodge in the walls of capillary beds in predilection sites such as the synovium, renal glomerulus, uveal tract and skin to establish an immune-complex vasculitis reaction. In this instance the inflammatory process (complement activation, leukocyte recruitment and activation) occurs within the vessel wall and endothelial damage may lead to local thrombosis and tissue ischaemia. Immune-complex disease is relatively common in veterinary medicine – but in the majority of cases, the identity of the initiating antigen is not known.

A range of factors will determine whether deposition of circulating immune complexes will occur in a particular vascular bed, including:

- size of the complex
- nature of the antigen
- nature of the antibody
- presence of increased vascular permeability with exposure of basement membrane permitting access for the complexes
- presence of high blood pressure and turbulent blood flow that marginalizes complexes to the vascular periphery.

The classical example of this form of type III hypersensitivity is 'serum sickness' that follows injection of a large dose of foreign serum (e.g. equine antiserum to tetanus toxin) creating the conditions of antigen excess.

Type IV (Delayed) Hypersensitivity

Unlike the former mechanisms, this manifestation of hypersensitivity involves mononuclear cells rather than antibodies. The immunological sensitization leads to generation of a population of antigen-specific Th1 lymphocytes, which upon subsequent exposure to antigen are activated and recruited into the tissue site of antigen exposure. These cells produce a range of cytokines (particularly IFN-γ) and chemokines (e.g. monocyte chemotactic proteins) which are responsible for modulation of local vascular endothelium and the recruitment of further mononuclear cells (chiefly macrophages and lymphocytes) into the inflammatory site. The network of pro-inflammatory and immunoregulatory cytokines produced by these cells is responsible for the clinical effects of local tissue inflammation. Because this cellular recruitment takes approximately 24-48 hours to occur, the type IV reaction is generally known as 'delayed type hypersensitivity' (DTH). The prototype DTH reaction is that which occurs in tuberculin testing. When mycobacterial antigen is injected into the skin, antigen-specific Th1 cells recognise antigen presented by APC and initiate the chain of events described above. As each stage of the response takes several hours, the optimum time for clinical readout of the lesions is 24 – 48 hours. The most common clinical example of such a reaction is contact allergic dermatitis.

Regulation of Hypersensitivity Reactions

The Gell and Coombs classification was developed many years before our current understanding of regulation of the immune response, but fits well with the modern concepts of regulatory T lymphocytes that drive most immune responses. For example, types I to III hypersensitivity would most likely be stimulated by activation of $CD4^+$ Th2 lymphocytes which secrete cytokines that drive humoral immune responses (i.e. IL-4, IL-5, IL-9 and IL-13). By contrast, type IV hypersensitivity is known to be stimulated by $CD4^+$ Th1 cells that preferentially produce IL-2 and IFN-γ. All hypersensitivity reactions would be inhibited by regulatory (suppressor) T lymphocytes producing IL-10. In fact, lack of such regulatory activity is proposed to be a major causative factor in the development of allergic disease – and immunotherapeutic approaches to allergy focus on restoration of impaired regulatory capacity.

The Benefit of Hypersensitivity Reactions

The hypersensitivity reactions are generally considered in terms of their involvement in the spectrum of allergic and autoimmune diseases of human and animal patients, and the prime clinical focus is in inhibiting such responses. In fact, in evolutionary terms – these mechanisms developed to provide selective survival advantage to animals that were increasingly exposed to an expanding range of pathogens. For example, the same IgE-mast cell type I response that underlies asthma or atopic dermatitis evolved to provide a beneficial immune response to parasitic infection and continues to do so today. Similarly, type IV reactions likely evolved to counteract infection with the intracellular pathogens (e.g. *Mycobacterium*, *Leishmania*) and it is well known that deficiency in this arm of immunity predisposes to such infectious disease.

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IMMUNOSUPPRESSIVE THERAPY IN THE DOG AND CAT

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There are numerous disease states in which the therapeutic approach centres on suppression of an over-active immune system (e.g. hypersensitivity, autoimmunity). Currently available methods to achieve immunosuppression are relatively crude medical approaches.

Immunosuppression is most commonly achieved with systemic administration of glucocorticoids, with or without concurrent adjunct agents. Glucocorticoids affect the function of immune cells, and produce a 'blanket immunosuppression' that inhibits the desired immune response (e.g. an autoimmune response), but which may also non-specifically inhibit protective responses to potential pathogens. Glucocorticoids are probably the single most commonly used drugs in veterinary medicine, but our understanding of the fundamental pharmacokinetics and immunological effects of these agents in dogs and cats is limited. Most of what we suppose these drugs to do *in vivo* is extrapolated from studies in man and experimental animals. Even the tapered dosage protocols that are used are 'borrowed' from human medicine and applied to dogs and cats in an empirical fashion. One of the few studies of the immunological effects of glucocorticoids in the dog examined a group of beagles given 2 mg/kg prednisolone SID for 14 days. The treated dogs showed reduction in the serum concentration of IgG, IgM and IgA, and reduced numbers of $CD4^+$, $CD8^+$ T cells and $CD21^+$ B cells. This investigation did not examine functional aspects of immunity, nor consider the effects of immunosuppression over the longer time periods that are generally used.

At higher doses, glucocorticoids are immunosuppressive and remain the drug of choice for management of autoimmune diseases. They affect the function of leucocytes including macrophages, and T and B lymphocytes. A range of glucocorticoids is used for immunosuppressive therapy, but the most common protocol involves prednisolone given at 2 – 4 mg/kg in divided doses daily in dogs; in cats possibly higher, but few good data support this. The glucocorticoid dose is reduced slowly over 8 – 10 weeks to achieve the lowest alternate day dose possible to prevent recurrence of clinical signs. The need for

combination therapy should be considered if remission is not achieved within 14 days or if side effects of glucocorticoids become unacceptable.

On the basis of clinical assessment of the severity of disease, other adjunct drugs are sometimes included into an immunosuppressive protocol. These agents include azathioprine, cyclophosphamide, danazol, ciclosporin, leflunomide, mycophenolate mofetil (MMF), clodronate, and in the cat, chlorambucil. The use of these drugs often does not have a good evidence-base and there has been empirical application of human protocols for companion animal immune-mediated disease. Many of these agents have well-recognized side effects and a 'blanket' immunosuppressive effect on the immune system. At least part of the rationale for the use of adjunct immunosuppressives is that they may permit a lower dose of glucocorticoid to be used in the maintenance phase of therapy – thus reducing the likelihood of steroid side effects.

Locally-delivered glucocorticoids (e.g. budesonide, beclomethasone, fluticasone) have been developed in an attempt to reduce systemic side effects. These drugs are particularly suitable for treating local inflammation at mucosal surfaces. Fluticasone administered by inhalation shows excellent tolerability in treatment of inflammatory airway disease in dogs. Clinical signs of secondary hyperadrenocorticism are not described and although there is some suppression of the hypothalamic pituitary adrenal axis (HPAA), this is much less marked than occurs with orally administered prednisolone. Budesonide has been used for treatment of canine inflammatory bowel disease (IBD) and does cause significant HPAA suppression. Prospective controlled studies on locally administered steroids in dogs are required.

The most significant recent evaluation of the clinical efficacy of these agents has centred upon their use in the management of canine IMHA. Several studies have suggested that combination prednisone and cyclophosphamide immunotherapy actually results in a poorer outcome than glucocorticoid monotherapy – and on this basis, the use of cyclophosphamide is not now recommended for treatment of the immune-mediated blood dyscrasias. The synthetic androgen danazol was also once widely used as an adjunct immunosuppressive and 'steroid-sparing' agent in the treatment of immune-mediated blood dyscrasias. Similar retrospective studies have now also shown that there is no additive beneficial effect on disease outcome, so use of this drug too is no longer

recommended. This essentially leaves only azathioprine as the remaining 'traditional' adjunct immunosuppressive.

Azathioprine is the most commonly used drug in combination protocols. It is converted by hepatic metabolism into the purine analogue 6-mercaptopurine and then to cytotoxic thioguanine nucleotides that suppress immune cell replication and response. Azathioprine is widely recommended for immune-mediated skin disease, haemolytic anaemia, immune mediated glomerulopathy, myasthenia gravis, rheumatoid and immune-mediated polyarthritis and inflammatory bowel disease. Its use has been associated with a number of side effects including myelosuppression, thrombocytopenia, hepatotoxicity, panniculitis, cutaneous drug eruptions, and commensal overgrowth. These effects are particularly pronounced in cats possibly due to limited hepatic detoxification of the drug. Consequently azathioprine should be avoided in this species or very low doses used. There may be a relatively slow onset of action of the drug and monitoring includes a full haematological and biochemical analysis every two weeks for the first 6 weeks of therapy at least. The immunosuppressive dose is 2.0 mg/kg once daily then on alternate days in combination with glucocorticoids.

MMF was developed as an alternative to azathioprine with reduced myelotoxicity and hepatotoxicity and is widely used in multidrug regimes for prevention of human allograft rejection and for treating several human immune-mediated diseases. MMF is metabolized 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 cytotoxic T cells and antibody responses are inhibited. MPA may also induce apoptosis in activated T cells and impair dendritic cell maturation. 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 2-4 hours after dosing and low toxicity with signs primarily limited to gastrointestinal effects, although mild suspected allergic reactions have been reported with the parenteral preparation. Anecdotally, usage of MMF for treatment of refractory immune-mediated disease is now widespread, especially for acquired myasthenia gravis, 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.

Chlorambucil is an alkylating agent that induces immunosuppression through its cytotoxic effects on the cell cycle of immune cells. It can be used instead of azathioprine in cats or in combination therapy with glucocorticoids or azathioprine /glucocorticoids in dogs. Its major side effects are bone marrow suppression and gastrointestinal disturbances. Its onset of action may be several weeks and the immunosuppressive dose is 0.1 – 0.2 mg/kg once daily or on alternate days.

Cyclophosphamide is a more potent alkylating agent than chlorambucil and is commonly used as a cytotoxic drug in chemotherapy. It may be used alone or in combination with glucocorticoids. Use may be associated with leucopenia, haemorrhagic cystitis and gastrointestinal signs. The immunosuppressive dose is: dogs >25kg, 1.5mg/kg on alternate days; dogs <25kg, 2mg/kg; dogs <5kg, 2.5mg/kg.

Leflunomide and its analogues exert their immunosuppressive effect by inhibiting lymphocyte and fibroblast proliferation. Leflunomide is licensed for treatment of human rheumatoid arthritis and has been used in some cases of steroid-resistant canine IMHA and a range of other autoimmune diseases. It has also been used to treat systemic histiocytosis in dogs. A dose rate of 4mg/kg over 24hrs with a blood level of 20 micrograms/ml has been recommended. The major signs of toxicity reported in dogs are gastrointestinal.

Ciclosporin was initially developed for human transplantation medicine and was subsequently used topically for the treatment of immune-mediated ophthalmic disease in the dog (e.g. KCS). The drug was originally used systemically in the dog for treatment of some immune-mediated diseases and anal furunculosis in a formulation that required regular monitoring of blood concentrations. However, there is now a licensed veterinary form of ciclosporin which is a microemulsion formulation that has greater bioavailability and more stable pharmacokinetics. Ciclosporin inhibits T lymphocytes by interfering with transcription of genes encoding a range of stimulatory cytokines, in particular IL-2. However, the drug is still relatively non-specific and will affect all T cells in a ‘blanket’ fashion. The consequences of T cell inhibition are lack of T cell help for B cell activation and antibody production. Ciclosporin is relatively expensive and has a range of side effects. Although currently only licensed for use in the treatment of atopic dermatitis ciclosporin is now widely used in the medical management of anal furunculosis and as an adjunct

immunosuppressant for other immune-mediated diseases. Combination glucocorticoid plus ciclosporin treatment for canine IMHA appears to offer little benefit over glucocorticoid monotherapy. Initial evaluation of this drug in the management of dogs with IBD has also produced equivocal results. There appears to be poor response in non-allergic immune-mediated skin diseases. Side effects relate to gastrointestinal irritation but the more severe signs of renal and hepatic toxicity seen in man are not seen in dogs. Recrudescence of subclinical *Toxoplasma* infection has been reported in cats. Several drugs interact with ciclosporin and many increase its blood levels making toxicity more likely. The related drug tacrolimus acts in a similar fashion to ciclosporin. This agent is available as a topical formulation that appears to have efficacy in the management of lesions of atopic dermatitis or the deep cutaneous sinus tracts of German shepherd dogs with generalized cutaneous or anal furunculosis.

A range of other agents is used for their anti-inflammatory or immunomodulatory effects – but again with a minimal evidence base. These include oral or injectable gold salts (chrysotherapy), megestrol acetate, pentoxifylline and tetracycline (or doxycycline)-niacinamide – the latter three drugs most commonly applied to dermatological disease. It is also likely that some of the antimicrobials commonly used in veterinary medicine (e.g. metronidazole, doxycycline) also have some immunomodulatory effects (based on studies in man and experimental animals) – but no such studies have been performed in dogs and cats.

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NOVEL IMMUNOTHERAPY

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Introduction

Immunotherapy is a broad term that covers means of either enhancing or suppressing immune function using a variety of novel approaches that are not necessarily mainstream pharmacological agents. The goal of immunotherapy is often to selectively modify one aspect of innate or adaptive immunity without having a blanket effect on the entire immune system. The scientific evidence base for immunotherapy is often not robust, but increasing numbers of published studies do now provide mechanistic support for some veterinary immunotherapeutic approaches.

Immunopotentiation

There are relatively few approaches to the non-specific enhancement of immunity in animals. Some of these are historical and no longer recommended. For example, the antiparasitic drug levamisole was once used to attempt to enhance canine immune function (specifically macrophage function) in dogs with deep pyoderma and was used in combination therapy for canine systemic lupus erythematosus. Such treatment sometimes led to the onset of cutaneous drug eruptions (specifically of the erythema multiforme type) and it is no longer recommended. A range of unlicensed bacterial extracts are sometimes used in dogs with deep pyoderma with view to enhancing the anti-bacterial immune response, but the evidence for efficacy is limited.

One product, Zylexis™ (formerly Baypamune™), is licensed in North America for the management of stress-associated acute respiratory disease in group-housed horses. The product consists of parapoxvirus ovis particles that are suggested to enhance innate immunity and there are now a number of published studies that show variable efficacy in this clinical situation. Zylexis™ also has a license for small animal use in some European countries.

Domperidone™ is a gastric prokinetic and anti-emetic drug that is now becoming used as an adjunct immunomodulator in the treatment of dogs with leishmaniosis. This

effect relates to the action of the drug as a dopamine D2 receptor agonist which leads to serotonin release and consequent prolactin production. Increased concentrations of serum prolactin are proposed to have a range of immunological effects, specifically in the enhancement of Th1-type immunity over Th2 responsiveness.

Allergen-Specific Immunotherapy

Allergen-specific immunotherapy (ASIT; hyposensitization) for canine atopic dermatitis appears to selectively target allergen-specific lymphocytes. Current research suggests that induction of regulatory T cells may be the major mechanism by which incremental dosage of allergen works. The newest approach to ASIT is sublingual delivery of allergen (sublingual immunotherapy; SLIT) which has been shown to have excellent efficacy. There is currently much research interest in the delivery of antigens (allergens or autoantigens or peptides derived from these molecules) across mucosal barriers (oral or intranasal delivery) with view to inducing immune 'tolerance' to the molecules and providing clinical benefit to patients. The delivery of allergens via 'gene therapy' with bacterial plasmids containing the allergen gene of interest has also been studied experimentally in a canine allergy model.

Cytokine Therapy

The use of recombinant cytokines is now well established in human medicine, where a wide range of such products exist. In veterinary medicine, cytokine therapy began with the use of human recombinant colony stimulating factors (e.g. Neupogen^{TM}) to enhance leucocyte production in neutropenic animals. Recombinant human anti-viral (type I) interferons have also been widely used parenterally or topically to attempt to treat a variety of feline viral infections (e.g. retroviral infection, FIP, upper respiratory tract viruses). More recently, the licensed recombinant feline interferon omega (Virbagen Omega^{TM}) has become available for the treatment of canine parvovirus infection and the adjunct management of feline retroviral infection. In Japan, recombinant canine interferon-gamma (Interdog^{TM}) is sold for the treatment of dogs with atopic dermatitis.

An alternative approach to recombinant cytokine therapy is delivery of the genes encoding target cytokines either by naked DNA transfer (bacterial plasmids) or using a recombinant vector organism. The latter approach is now available in veterinary medicine

with the launch of Oncept IL-2^{TM}; the canarypox vector carrying the feline IL-2 gene. This product is licensed for the adjunct management of the feline injection site sarcoma and is delivered by repeated intra- and perilesional injection in conjunction with radiotherapy and following surgical excision.

Intravenous Immunoglobulin Therapy

High dose intravenous human immunoglobulin therapy has been used successfully in the management of challenging cases of canine immune-mediated haemolytic anaemia (IMHA) and thrombocytopenia (IMTP) and in some cases of immune-mediated skin disease. In IMHA and IMTP, the human protein acts by blocking macrophage Fc receptors and inhibiting phagocytosis of antibody-coated erythrocytes or platelets. In other immune-mediated disorders, the mode of action might be by enhancing the activity of regulatory T cells.

Monoclonal Antibody Therapy

In human medicine there are now numerous monoclonal antibody-based products that are injected intravenously to target specific molecules in cancer or immunological or inflammatory pathways. Monoclonal antibodies may be used as drug or prodrug delivery vehicles ('magic bullets') or to block or delete particular molecules or cells. The most widely used of such products in human medicine is Infliximab^{TM}, a monoclonal antibody specific for the cytokine tumour necrosis factor (TNF)-α. The antibody targets and neutralizes this proinflammatory cytokine in patients with a variety of immune-mediated diseases including psoriasis, rheumatoid arthritis, multiple sclerosis and inflammatory enteropathy. One study has applied the human product to management of a colony of dogs with cutaneous lupus erythematosus. A feline anti-TNF-α panel has been produced with view to the treatment of cats with FIP infection. There are now several biotechnology companies that are producing and licensing monoclonal antibody therapies for small animals. For example, Aratana Therapeutics has licensed monoclonal antibodies for the treatment of canine T- and B-cell lymphomas.

Apoquel^{TM}

This newly released drug acts to inhibit the Janus Kinase (JAK) pathway of signal transduction and thereby block the ability of cells to activate specific genes. The drug is

licensed for the treatment of allergic skin disease and the proposed major mode of action is blockade of the ability of lymphocytes to produce the cytokine interleukin (IL)-31. IL-31 is proposed to be one means by which cutaneous nerve endings are activated leading to CNS signalling and induction of the 'itch-scratch cycle'. The clinical effect of the drug is anti-pruritic and there is now a large body of immunological and clinical literature supporting the efficacy and proposed mode of action.

Adjunct or Alternative Immunotherapy

A wide range of approaches have been studied in attempts to boost immune function in people and animals. There is an entire field of research that aims to identify dietary supplements that might enhance immunity and numerous such studies have been conducted in small animal medicine. The use of prebiotics or probiotics aims to boost the function of regulatory T cells induced via the intestinal mucosa. The most interesting such approach also exploits the intestinal mucosa as a potent means of influencing systemic immunity (and particularly Treg activation). The deliberate establishment of an intestinal parasitic infection to modulate systemic immune function has been the subject of a series of human clinical trials and one reported study examined this approach in the management of dogs with atopic dermatitis.

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ADVANCES IN VACCINE TECHNOLOGY

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Introduction

Vaccination is the most high profile clinical application of immunology. Companion animal vaccination has been highly successful over the past five decades, such that in many western countries the prevalence of canine and feline infectious diseases remains relatively low. Indeed, in circumstances where vaccine uptake in a population decreases it is well-documented that infectious diseases (e.g. canine distemper or parvovirus) will re-emerge. Current vaccine technology is largely based on the technology of the 1960s and, with few exceptions, most licensed veterinary vaccines are live attenuated (infectious) or killed adjuvanted (non-infectious) products. However, we are beginning to see a number of changes in vaccine technology which are the subject of this presentation. Many of these are based on recent advances in our understanding of fundamental immunology – in particular the role of particular T cell subsets and cytokines in mediating protective immune responses to different classes of pathogen. Although these developments are exciting, it may take some time before products based on new technology come to market. The licensing of new products, particularly those based on molecular technology, is complex, time consuming and expensive – and the desire for such products must be balanced against the relatively small market represented by veterinary sales.

Modification of existing technology

Although current vaccine products might be rooted in traditional methods of production, these are all efficacious products with well-documented safety. Manufacturers have not been complacent and are quick to react to the demand for improvement or modification to products on the basis of the changing pattern of infectious disease in the population. One major challenge in this regard is the lack of substantial co-ordinated surveillance programmes for small animal infectious disease (other than those of direct zoonotic importance). Despite this limitation, there have been changes in product composition in

order to 'keep pace' with field changes. Examples of such change would include the introduction of multivalent *Leptospira* vaccines, ensuring cross-protection against emerging biotypes of canine parvovirus and development of bivalent feline calicivirus products.

In addition to efforts to maintain efficacy, manufacturers have also reacted responsibly to calls for increased vaccine safety. Much recent attention has been paid to the range of relatively uncommon adverse effects associated with vaccination, in particular those linked to the use of adjuvanted products. Consequently, another change has involved the development of non-adjuvanted vaccines – particularly for use in the cat. More importantly has been the introduction of products licensed with an extended duration of immunity (to three or four years) which enable the development of vaccine protocols whereby not all vaccine components need be administered every year. Associated with this has been the success of re-education of the veterinary profession regarding vaccination, and the introduction of the concept of 'annual health checks'. Professional organizations (e.g. AAHA, AVMA, AAFP and WSAVA) have provided leadership in this area with the production of up-to-date vaccine guidelines for the dog and cat.

HOW IS VACCINATION ACHIEVED?

Vaccination (as we currently use it) involves the administration of a microbial antigen, generally in advance of natural exposure to infection, to induce an immune response and immunological memory. The immunological mechanisms underlying the development of protective immunity have been described separately.

BASIC REQUIREMENTS OF A VACCINE

In practical terms the ideal vaccine would be cheap to produce and administer in order to maximize uptake amongst a population. The product would be stable (i.e. have a long shelf life without requiring a cold chain) for ease of use in the field, and would lack any side effects.

Immunologically, the ideal vaccine would induce a potent memory immune response that would persist over a long period of time (long duration of immunity). Moreover, the nature of the immune response induced would be appropriate for the infectious agent – i.e. would induce either or both humoral and cell-mediated immunity where required and therefore be of a type that was able to protect the animal from challenge with virulent

organism (long duration of protection). In terms of the basic immunological mechanisms described above, an ideal vaccine would:

* Be administered via route that mimicked that for natural exposure to the organism
* Be readily taken up by antigen presenting cells for antigen processing and presentation
* Be able to stimulate functionally appropriate populations of antigen-specific T and B lymphocytes specific for a range of protective epitopes
* Be capable of inducing persisting memory, perhaps by retention of small quantities of the antigen within lymphoid tissue.

TYPES OF VACCINE

Vaccines designed to protect from infectious disease now come in a range of different forms. The application of molecular biology to vaccine production has resulted in a new generation of products that are now used in day-to-day veterinary practice.

Live Organisms (Infectious Vaccines)

Live organisms incorporated into vaccines may be virulent (rarely), attenuated or heterologous. One veterinary example of vaccination based on a virulent organism is that used to control contagious ecthyma (orf) in sheep, which involves the scarification into the skin of infected scab material. The most common form of live organism vaccine involves attenuation of the organism such that the organism has reduced virulence and cannot cause overt disease. There are a variety of means of achieving attenuation, including:

* heating to below thermal death point
* exposure to sub-lethal concentration of chemicals
* adaptation to growth in unusual conditions, or in cells or species to which not normally adapted.

Finally, it is possible to utilize antigenically-related 'heterologous' organisms that will induce protective immunity but not disease in a specific species. An example of this approach is the use of measles virus to protect dogs from distemper. It is important that the organisms incorporated into vaccines are relevant to the field strains of infection that are currently present within a particular geographical area. There is particular discussion of this point with respect to canine *Leptospira* and parvovirus and feline calicivirus vaccines.

Live organism vaccines have numerous advantages over killed versions. These include:

* Live organisms should induce more potent immunity as they may still infect, replicate and release antigenic substances. Live organisms may also migrate from the site of injection to anatomical sites relevant to the infectious agent – ensuring local protective immunity.
* Live organisms should require fewer doses to achieve the desired response.
* Live organisms generally do not require adjuvant.

However, there are also particular disadvantages to live organisms, including:

* A possibility of 'reversion to virulence' – i.e. loss of attenuation and production of disease
* A greater possibility of contamination during production by other agents
* A lower stability that generally requires cold chain storage.

Dead Organisms (Non-Infectious Vaccines)

The alternative approach to whole organism vaccination is to utilize a killed organism that is unable to replicate or induce disease but is antigenically intact and able to stimulate the host immune response. A range of means of killing organisms has been used, but most involve chemical inactivation with substances such as formaldehyde, alcohol or alkylating agents. The major advantage of killed organism vaccines is their stability and the fact that they are relatively economical to produce. However, for reasons outlined above, they are less potent and generally require multiple doses and to be given in adjuvant. Because of the nature of the adjuvants most widely used in vaccine production (e.g. alum), such vaccines are more likely to induce a Th2 immune response characterised by antibody production, rather than strong cell-mediated immunity. Adjuvant is also deemed as being less than desirable, as these substances may be associated with side effects.

Metabolic or Structural Products from Organisms (Subunit vaccines)

This type of vaccine contains specific metabolites or structural proteins from an organism rather than the entire microbial particle. Production of this type of vaccine necessitates knowledge of the most relevant antigenic epitopes carried by an organism that are likely to

induce protective immunity. An example of such a product is the Leukocell FeLV vaccine that contains FeLV gp70 isolated from cell culture.

Synthetic Peptide Antigens

Synthetic peptide vaccines take the subunit approach one step further and utilize synthetically produced epitopes rather than native molecules obtained by fractionating an entire organism. These vaccines generally require adjuvants and are often of low immunogenicity.

New Vaccine Technology

Genetically Modified Organisms

The insertion of genes encoding specific antigenic epitopes into vector organisms is already a reality, with the commercial availability of recombinant canarypox-vectored FeLV, CDV and rabies vaccines. The same vector has been used to produce an experimental feline vaccine against highly pathogenic avian influenza virus. It is theoretically possible to enhance the effectiveness of such products via the use of molecular adjuvants. For example the incorporation of genes encoding particular host species cytokines into the vector organism might direct that a specific functional type of immune response is made to the vaccine. Inclusion of genes encoding the cytokines IL-12, IL-18 or IFN-γ might for example engender a strong cell-mediated (Th1) immune response.

Naked DNA vaccines

The next stage in the development of molecular vaccines involves a similar approach to that described above, but without the use of carrier organisms. The gene of interest is inserted directly into a bacterial plasmid and the plasmid DNA may be injected directly to the recipient animal via a number of routes including IM, SQ, percutaneously (may be injected at high velocity using purpose-designed apparatus) or fed orally (coupled to protective inert carrier). In this instance, the gene 'transfects' host cells, particularly antigen presenting dendritic cells, resulting in peptide expression on the surface of the cell associated with histocompatibility complex molecules. These APCs migrate to regional lymphoid tissue to induce immune response.

This has been shown to be a particularly powerful means of inducing protective immunity, as both strong humoral and cell-mediated immune responses are made. Experimentally, naked DNA vaccination has been shown to work in dogs and cats with rabies, distemper and FIV viral genes. There are some licensed veterinary products which already employ this technology (e.g. a vaccine for equine West Nile virus infection). The approach might induce immunity in the face of maternal immunoglobulin which has clear attraction for field use. Moreover, DNA vaccines are very stable and do not require cold-chain storage. The most recent reports of the use of naked DNA vaccines for rabies virus infection have shown that a single intradermal injection into the skin of the pinna induces protective immunity that is active in dogs challenged with virulent rabies virus one year after vaccination. Moreover, similar means of adjuvanting these vaccines by incorporation of cytokine genes or immunostimulatory bacterial CpG motifs into the plasmid have been tried. Such approaches must be introduced cautiously, as the desired effect is not always obtained. The latter substances are a specific target for those dendritic cell pattern recognition receptors that result in the induction of Th1 (cell-mediated) immune responses. CpG motifs have proven ability to redirect the canine immune response as they have been utilized to alter immune function in allergen-specific immunotherapy of canine atopic dermatitis and enhance responses to rabies vaccine.

Marker vaccines

Marker vaccines are a recent advance in vaccinology that enable a distinction to be made between an animal that is seropositive to a particular infectious agent because it has been vaccinated, compared to one that is seropositive because it has been infected with virulent field organism. There are numerous situations in which this distinction is crucial – for example in the UK this was one reason why vaccination was not used in the 2001 outbreak of foot and mouth disease. A marker vaccine has recently been introduced to enable distinction of cattle vaccinated for IBR. The vaccine is based on a glycoprotein E negative mutant of the virus, so a cow that has serum antibody to this glycoprotein must have been naturally infected. The use of marker vaccines necessitates parallel development of appropriate diagnostic tests to enable the distinction to be made. In companion animal medicine, there is a need for marker vaccines. For example, seropositivity to *Borrelia* in dogs might reflect vaccination rather than field exposure – although the use of western

blotting can determine between these possibilities as vaccinated animals make a strong antibody response to the OspA protein. Moreover, the new Snap4DX test utilises an antigen that appears to be specific for field infection. Another example is the FIV vaccine which induces serum antibody equivalent to field exposure. A marker vaccine would help distinguish between these possibilities.

New delivery routes

Alternative routes of vaccine delivery (e.g. oral, topical percutaneous, intranasal) are currently receiving much attention. In companion animal vaccination we already have intranasally-administered products, an oral vaccine and a vaccine that is delivered percutaneously via high pressure 'needle free vaccination'. It is likely that further developments will occur in this area. There is a particular focus on mucosal delivery of vaccines and the generation of 'mucosal adjuvants' such as cholera toxin or the B subunit of *E. coli* heat labile enterotoxin. Similar investigations are reported into the application of novel vehicles for vaccine delivery. Molecular vaccines have been incorporated into plants such as bananas or potatoes – and such 'edible vaccines' have enormous potential in the third world. Such a feed-delivered product already exists for use in poultry. Experimental studies have examined targeted lymph node delivery of vaccination or the *in vitro* uptake of antigen by cultured dendritic cells which are subsequently reinfused into an animal. Such approaches directly bypass the uncertainties of dendritic cell uptake of antigen when injected into the subcutaneous or intramuscular environment.

New vaccination protocols

There have already been many recent changes in the protocols by which we vaccinate our companion animals (see above). One such future development might include the introduction of the 'prime-boost' method in which a DNA and protein or vectored vaccine are given alternatively to engender a more potent immune response than can be achieved by two injections of either vaccine type alone.

Vaccination for allergy

We have been using allergen-specific immunotherapy for the management of canine and feline atopic dermatitis for many years. The repeated injection of aqueous or alum-

precipitated allergens by prolonged or 'rush' protocols is of clinical benefit in up to 80% of canine patients – but the immunological basis for the effect is poorly understood. Numerous advances are predicted in this field in the future.

We are already developing more detailed understanding of the precise allergenic epitopes to which our patients respond, which will allow the development of immunotherapy using purified, recombinant peptides rather than crude whole allergen preparations. Delivery of these allergens together with molecular adjuvants or via novel routes (see above) will likely increase efficacy. In an experimental model of feline flea allergy dermatitis, therapeutic benefit was seen following immunization with recombinant flea salivary protein combined with plasmid DNA containing the gene encoding the protein. This protocol induces regulatory T cells in the allergic animals. Vaccination with recombinant IgE peptide fragments to induce neutralizing anti-IgE antibodies in dogs has also proven possible.

Vaccination for autoimmunity

In human medicine a number of clinical trials are currently in progress examining the therapeutic potential of peptide vaccination for patients with autoimmune disease. The immunodominant peptide epitopes derived from target autoantigens have been defined and used to selectively stimulate 'suppressive' immune responses involving the induction of IL-10 producing T regulatory cells. For example, patients with multiple sclerosis are being treated with immunodominant peptides from myelin protein delivered across the nasal mucosa which is a potent means of stimulating such tolerance responses to autoantigens.

Vaccination for cancer

Similarly, there are numerous research groups and biotechnology companies developing immunotherapeutic vaccines for the treatment of patients with cancer. Much work has been done with melanoma in which target 'tumour antigens' to which the immune system naturally responds have been characterized. One such target antigen is the molecule tyrosinase, and in 2006 there was conditional licensure for the very first canine cancer vaccine which is based on a plasmid incorporating the human tyrosinase gene. Delivery of this vaccine will result in transfection of patient dendritic cells and stimulation of an immune response against the tumour antigen. The adjunct therapeutic use of recombinant IL-2

(delivered via the canarypox vector) in feline injection site sarcoma is another advance in this area.

Other applications

The potential applications for the process of vaccination in the future are broad. Current research is investigating the development of vaccines for parasite infestation and subcutaneous injection of dogs with L3 larvae of *Ancylostoma caninum* has been shown to induce strong Th2 immune responses and reduce the severity of challenge infection. Other studies are seeking to develop immunocontraceptive vaccines by injection of dogs with synthetic peptide constructs of the 10 amino acid long luteinising hormone releasing hormone (LHRH). In these studies the LHRH peptide (a B cell epitope) is linked to a peptide sequence derived from the fusion (F) protein of CDV which should act as a T helper epitope in previously vaccinated dog.

Vaccinomics

New developments in vaccination will come not from just advances in formulating vaccines, but from understanding more completely the host immune response to vaccination. Vaccines may be tailored to individuals with a specific genetic background as determined by genome wide screening studies.

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VACCINATING DOGS AND CATS: WHAT IS ESSENTIAL?

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COMPANION ANIMAL VACCINATION HISTORY

Vaccination of dogs and cats to protect from infectious disease has been practised globally since the 1960s. In developed countries vaccination has proven highly successful in maintaining control of life-threatening viral diseases caused by canine distemper virus (CDV), canine adenovirus (CAV), canine parvovirus (CPV) and feline parvovirus (FPV). Vaccination has also had major impact in reducing morbidity and mortality associated with infections caused by feline calicivirus (FCV), feline herpesvirus (FHV), canine parainfluenza virus (CPi), feline leukaemia virus (FeLV), *Chylamydophila felis*, *Bordetella bronchiseptica* and *Leptospira interrogans* and new infectious disease vaccines continue to be developed (e.g. feline immunodeficiency virus [FIV], canine influenza virus [CIV]). In endemic areas, rabies virus vaccination of companion animals has major impact on the prevalence of this zoonotic infection in the human population. When used in co-ordinated control programmes vaccination has led to elimination of two infectious diseases globally – human smallpox (1979) and bovine rinderpest (2011). We are far from eradicating companion animal infections, which remain highly prevalent in developing countries and emerge as localized outbreaks in even the most developed nations.

Although vaccination is now well entrenched in companion animal practice, as for many medical and surgical procedures, vaccination theory and practice continues to evolve. This presentation reviews the changes in companion animal vaccination that have occurred over the past decade to give the basis for 21st century protection of the dog and cat populations.

WHAT HAS TRIGGERED CHANGE?

There are striking parallels in recent changes in vaccination practice in human and veterinary medicine. Attention has focussed over the past two decades on issues related to the safety of human and companion animal vaccines. Within the veterinary profession the discussion over vaccine safety first emerged following the 1989 publication that suggested

that some aggressive cutaneous sarcomas in cats might be related to vaccination. The feline injection site sarcoma (FISS) remains a serious problem in many countries today. In 1996 attention was focussed on the possibility that vaccination might trigger autoimmunity in dogs and initial reports of vaccine-associated immune-mediated haemolytic anaemia (IMHA) and thrombocytopenia extended to cases of polyarthritis or glomerulonephritis triggered by vaccination and the identification of autoantibodies specific for thyroid proteins in dogs and renal tubular epithelial cells in cats.

In parallel with these observations has been the enormous media attention given to human adverse reactions post-vaccination; in particular the now refuted claims that childhood vaccination might be associated with autism and Crohn's disease. New human vaccine scares continue to emerge with the introduction of new products (e.g. the human papilloma virus vaccine for cervical cancer and the potential association between narcolepsy and H1N1 influenza vaccination). The airing of these claims in the media and through the internet has led to inevitable parallels being drawn between human and companion animal adverse reactions. In many countries influential public lobby groups have become prominent in providing web-based opinion (and often misinformation) on companion animal vaccination – and it is all too common for these concerns to be raised in the consultation room. The phenomenon of internet 'vaccinophobia' is now being studied and recorded in the scientific literature.

So are companion animal vaccines safe? The first fact to recognize is that no human or animal vaccine can be guaranteed 100% safe in any individual – but vaccines are very safe products with a low prevalence of associated adverse reactions. The high safety of vaccines is a reflection of the rigorous safety testing that must be undertaken before any product comes to market. It is difficult to put precise figures on the prevalence of adverse reactions to vaccines in dogs and cats. Some data are available from governmental pharmacovigalence schemes and the published scientific literature and when all reactions are considered (from common mild and transient illness to rare instances of death) the figure is somewhere between 0.61 to 38 reactions per 10,000 doses of vaccine (sold or administered, respectively) for dogs, and 0.21 to 51.6 reactions per 10,000 doses of vaccine (sold or administered, respectively) for cats.

Even though adverse reactions are uncommon, best practice medicine should dictate that we make every effort to reduce further the prevalence of such reactions, for the benefit of companion animals and their owners.

HOW HAS THE PROFESSION RESPONDED?

The veterinary profession has responded in an appropriate and responsible manner to concerns over vaccine safety and the prevalence of adverse reactions. Expert groups have been established and these have produced information and guidelines to allow practitioners to develop and implement safer and more scientifically robust vaccination practices. Guidelines have been produced by the American Association of Feline Practitioners (1998, 2000, 2006 and 2013), the American Animal Hospital Association (2003, 2006 and 2011), the World Small Animal Veterinary Association (2007, 2010 and 2015) and the European Advisory Board on Cat Diseases (2006 and 2013). These various guidelines are essentially similar in their recommendations and those from the WSAVA have had greatest global impact. Many national veterinary associations have endorsed the WSAVA guidelines or used them to develop their own national recommendations.

There is often confusion over guidelines as in some instances they appear to conflict with recommendations given in the data sheets (or summary of product characteristics) that is the legal document that accompanies a vaccine. Put simply, guidelines are a reflection of current scientific thinking and offer advice to practitioners to help them use vaccines in the safest and most immunologically correct fashion. The advice given in guidelines is generally more current than that in data sheets, which for older products may not have been updated since initial licensure. Guidelines are not compulsory and have no legal standing. They may need to be adapted to local circumstances and are simply a tool for practitioners to use in developing a practice vaccination strategy. Where guideline recommendations do differ from those on data sheets (this is increasingly less common), then the application of guidelines recommendations (off-label use) should be done with informed client consent.

The WSAVA guidelines are readily accessible from the WSAVA website, where you may also find a substantial information document written for pet owners and breeders and a series of single-page infectious disease fact sheets that are designed for use in the consultation room setting. What follows is a distillation of guideline recommendations given as 10 fundamental concepts in vaccinology.

1. WE SHOULD VACCINATE MORE ANIMALS

One of the major recommendations of the WSAVA guidelines is that as many animals as possible in a national or local population should be vaccinated. This comes from the concept of **herd immunity** which very simply states that where at least 75% of a population is vaccinated, it becomes very difficult for particular infectious agents to cause disease outbreaks in that population. There are clear examples of the importance of herd immunity in human and companion animal populations – where vaccination uptake has declined, allowing the re-emergence of previously controlled infections. It is best-practice medicine to ensure that as many dogs and cats in your practice area are vaccinated. Recent reports have suggested that one of the impacts of the current global economic recession has been reduced vaccination uptake by dog and cat owners – leading to concerns over the potential for disease outbreaks.

2. VACCINES ARE CORE OR NON-CORE

All vaccination guidelines categorize vaccines as **core** or **non-core**, which provides a framework for considering how these different products might best be used. Core vaccines are those which every dog or cat should receive to protect them from diseases that are life-threatening or that cause significant morbidity. Core canine vaccines for dogs are those that protect against CDV, CAV and CPV and for cats FPV, FCV and FHV (and for both species, rabies in endemic areas). Non-core vaccines are those that are only required by some dogs and cats, where their lifestyle or geographical location puts them at risk of contracting disease.

3. NON-CORE VACCINES SHOULD BE SELECTED ON BENEFIT-RISK ANALYSIS

Which vaccines are non-core may therefore differ from country-to-country, from state-to-state or even town-to-town. Practitioners must assess available information on local disease prevalence and take into account the lifestyle of the individual animal when deciding on the use of non-core vaccines. This means a fundamental change in the delivery of vaccination, in which there is a move away from the 'practice vaccination policy' (the same protocol applied to every dog or cat that walks through the door) to '**individualized medicine**' related to the needs of that individual animal.

4. CONTRIBUTE TO DISEASE SURVEILLANCE

In human medicine, informed decision on the use of non-core vaccines can be made on the basis of disease surveillance data that precisely maps where particular diseases are prevalent. Sadly, we lack such data in companion animal medicine, but there are some national voluntary disease reporting schemes that collate and publish disease distribution maps for the key canine and feline vaccine-preventable diseases. It should be beholden on all veterinarians to contribute information into these databases for the benefit of our animals and their owners.

5. CORE VACCINES SHOULD BE GIVEN NO MORE FREQUENTLY THAN EVERY 3 YEARS

This fundamental change is one of the key platforms of vaccination guidelines. When triennial revaccination of adult animals was first recommended, all vaccine products had a 1-year minimum licensed **duration of immunity** (DOI) and so this shift was regarded as contentious and treated with suspicion. A decade later, in the USA and Europe virtually all core canine MLV vaccine products and all FPV products had a 3-year minimum licensed DOI and triennial vaccination is now the norm. So what is DOI and how does it influence the choice of revaccination interval?

DOI is simply the time after vaccination that an immune response (generally the presence of serum antibody to the vaccine antigens) can be detected. The DOI varies between different vaccines. Serum antibody titres decline relatively rapidly after vaccination with *Leptospira* or rabies, but persist after vaccination with MLV CDV, CAV, CPV or FPV. A second term '**duration of protection**' (DOP) is used to describe the time after vaccination that one can challenge an animal with a virulent organism without it developing disease (or pathology, or death depending on the claim for the vaccine). An animal may lack serum antibody, but still have effective cellular immunity and immunological memory and so be protected from disease. For FHV infection, cellular immunity provides a better correlate to protection than serum antibody. Similarly, for intranasally-administered CPi and *Bordetella* products, local mucosal antibody (particularly IgA antibody) is a more relevant correlate of protection than serum antibody concentration and mucosal immunity may also correlate better with protection against FCV. Although DOI and DOP are technically different terms, we often use DOI in reference to protection.

Vaccine licensure requires that the manufacturer provides information on the **MINIMUM** duration of immunity of the vaccine. Traditionally this has been for a 1-year period, which is why vaccines were licensed with a 1-year DOI. As stated above, most manufacturers have now provided further experimental data that support a **MINIMUM** duration of immunity of at least 3 (and sometimes 4) years. These new studies are generally done with the identical (or very similar) vaccines – it is not that some wonderful new product has been developed that provides longer-lasting immunity. MLV core vaccines with a 1-year licensed DOI have always been able to provide long-lived (and probably lifelong) immunity. For that reason, even in countries where core MLV vaccines still have only a 1-year licensed DOI, they can effectively be used in triennial programmes. Of course, where licensed products carry a 3-year DOI, it becomes 'off-label' to use them annually and such usage should only be with informed client consent.

But if 3 years is the MINIMUM duration of immunity – what is the maximum? In reality, core MLV vaccines (CDV, CAV, CPV and FPV) probably provide animals with lifelong immunity following appropriate puppy or kitten vaccination (see below). There are two forms of evidence that support a longer DOI than that currently accepted globally as minimum (3 years). The first is serological. The presence of virus neutralizing serum antibody is a strong '**correlate of protection**' for CDV, CAV, CPV and FPV and WSAVA guidelines indicate that the presence of antibody at any titre equates to a protective immune response and the presence of immunological memory. Numerous experimental and field studies have shown that animals vaccinated as pups or kittens (and not routinely as adults) remain seropositive for up to 14 years and there are new Australian field data showing protective titres in dogs last vaccinated >42 months previously (and up to 9 years previously). More importantly, there are **experimental challenge data** that show robust protection on challenge of dogs last vaccinated up to 9 years previously with core canine vaccines, and up to 7.5 years previously with core feline vaccines. Whether in future canine and feline vaccines will carry even longer licensed DOI depends upon whether manufacturers are able (financially and ethically) to make the large investment required to provide experimental challenge data.

6. ENSURE PROTECTION OF PUPS AND KITTENS

The reasoning behind the use of multiple vaccines in primary vaccination of puppies and kittens is a fundamental immunological principle related to the blocking effects of **maternally-derived antibody** (MDA) against vaccine antigens. It is not possible to predict for any one animal when the 'window of susceptibility' (where there is no longer sufficient MDA to provide adequate protection, but still sufficient MDA to block endogenous response to vaccination) might lie. Veterinarians have become comfortable with vaccinating at 8 and 12 weeks assuming that MDA will have degraded in all pups and kittens by 12 weeks of age. New data suggest that higher-titre vaccines increase the concentration of MDA, which persists for a longer period. These data suggest that at 10 weeks of age, only 75% of pups are capable of responding to CPV vaccine and that at 12 weeks of age, only 90% of pups can respond. In some kittens, MDA has been shown to persist to up to 20 weeks of age. For this reason we now recommend that a third vaccination in the puppy and kitten series be given at 16 weeks or older, when all animals should be able to respond. Importantly, the puppy or kitten vaccination schedule must include either a 26 or 52 week booster vaccine.

The guidelines advice on puppy vaccination is at odds with the introduction of 'early finish products' that are designed to be given at 8 and 10 weeks of age to permit early socialization of puppies. Guidelines groups recognize the importance of early socialization for the behavioural development of young animals, but caution that there is an element of risk in this practice.

7. ADULT ANIMALS MAY BE VACCINATED EVERY YEAR – BUT JUST NOT WITH EVERYTHING!

There is a common misconception that guidelines advice is that animals may not be vaccinated every year and that this may therefore reduce veterinary visits and impact on practice income. In fact, in most situations, dogs and cats will still receive an annual vaccination – but just not with all components. This situation arises because most non-core vaccines retain a 1-year DOI and cannot be used effectively at intervals of greater than 1 year. So in reality, while an adult dog may only receive MLV CDV, CAV and CPV every 3 years, in the intervening years the animal may receive non-core vaccines depending on that animal's benefit – risk analysis (and providing that there are product ranges available that contain the appropriate monovalent or combination antigens). Although all three core feline MLV vaccine components (FPV, FCV and FHV) are recommended for triennial use, only

the FPV component has a triennial license in some countries. One product has recently been licensed with a 3-year claim for FCV and FHV. It is recognized that the lifestyle of some cats (e.g. multicat households or frequent cattery visits) may increase the risk for upper respiratory tract infection and so benefit – risk analysis in these animals suggests that it is acceptable to give annual FCV and FHV vaccination (again providing that suitable product ranges exist). Whichever schedule is chosen, the important concept is that reduced numbers of vaccine antigens are being delivered to that animal overall, thereby increasing the margin of safety.

8. MINIMIZE USE OF ADJUVANTED VACCINES IN CATS

Although it is now clear that FISS may be linked to a wide range of injectable products, there is still evidence that the local inflammatory response that precedes neoplastic transformation may be more intense in the presence of adjuvant. Non-adjuvanted feline vaccines should be used where available. Advice varies on the optimum site of vaccination for cats and the distal limbs, lateral abdomen and distal tail are all suggested. At very least vaccines should not be administered into the scruff and the site of administration should be rotated (and recorded) on each occasion.

9. USE SEROLOGICAL TESTING TO AID DECISION MAKING

Until recently, determining whether an animal had serum antibody specific for vaccine antigens required sending a blood sample to a specialist diagnostic laboratory for virus neutralization (CDV, CAV, FCV, FHV) or haemagglutination inhibition (CPV, FPV) testing. Simple in-house test kits are now available that can determine the presence of serum antibody to CDV and CPV (Synbiotics Titerchek™) or to CDV, CAV and CPV (Biogal Vaccicheck™) or to FPV, FCV and FHV (Biogal Feline Vaccicheck™). There are multiple uses for such kits in a vaccination programme to assist with decision making on vaccination and to reduce vaccine load in an individual animal. Such kits are now well-entrenched as part of the practice laboratory in the USA and are becoming popular in Europe. Informed pet owners may on occasion request serological testing instead of routine revaccination.

The canine kits may be used to determine whether a pup has responded to primary vaccination by testing at 20 weeks of age. This is particularly relevant for some low-responder breeds (e.g. rottweilers) where alternative strategies might be implemented on

the basis of the test result. Serological testing may also be invaluable in determining whether an animal that has previously suffered an adverse reaction to vaccination (e.g. a dog with vaccine-associated IMHA) requires revaccination that may trigger a further episode of disease.

Serology may be used to determine the best vaccination protocol for an adult dog with 'lapsed' vaccination or of unknown vaccination history. A seropositive adult dog does not require core vaccination and may move to a triennial revaccination programme. A seronegative adult dog should only require a single dose of core MLV vaccine with triennial boosters. For non-core vaccines, two doses and then annual boosters are required.

In the context of the annual health check (see below), serology may be used routinely at the time of the annual veterinary visit in order to determine vaccine requirements for the coming year. The aim of this is simply to avoid unnecessary revaccination and minimize the risk of adverse reactions.

10. VACCINATION SHOULD BE DELIVERED AS PART OF AN ANNUAL HEALTH CHECK PROGRAMME

All of the above concepts are enshrined in the '**annual health check**'. The annual health check programme was developed in the USA and is now becoming increasingly popular in Europe. The annual health check is a new means of marketing and promoting veterinary services. There is a move away from using vaccination as the primary reason for an annual veterinary visit, towards a professional consultation that considers equally all aspects of the health and well-being of the animal. The use of 'annual booster' terminology and reminder cards should be replaced by reminders to visit for the annual health check. Vaccination becomes just one part of a discussion between the veterinarian and client. Decisions about vaccination are made on the basis of the lifestyle of the individual (individualized medicine) and are reviewed annually, perhaps with the aid of serological testing. There is no longer a 'one size fits all' vaccination regime. The prominence of vaccination might also be reduced in invoicing – the client should not be paying for the vaccine or vaccination *per se*, but for the professional consultation. The vaccine is often provided at cost price (or even for free) in many programmes where the professional fee is the top line. None of this threatens practice income – it is the presentation and marketing that changes – and where used appropriately, practice income may actually benefit.