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Review article

Mucosal defence along the gastrointestinal tract of cats and dogs

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Abstract – Diseases that are associated with infections or allergic reactions in the gastrointestinal and respiratory tracts are major causes of morbidity in both cats and dogs. Future strategies for the control of these conditions require a greater understanding of the cellular and molecular mechanisms involved in the induction and regulation of responses at the mucosal surfaces. Historically, the majority of the fundamental studies have been carried out in rodents or with tissues obtained from man, but the expanding range of reagents available for the study of farm and companion animals provides opportunities for study in a wider range of animals including cats and dogs. To date, these studies have tended to be focussed on characterising the cellular distributions in healthy animals and in groups of cats and dogs identified as having an increased risk of mucosal disturbance. Where species comparisons of mucosal immune systems have been made, the results have tended to be divided between monogastric and ruminant animals. It is then not surprising that the mucosal immune systems of both cats and dogs bear greatest similarity to that documented for man and pigs. For example, IgA is the dominant immunoglobulin in mucosal secretions of cats and dogs and oral tolerance can be induced following the introduction of novel antigens into the diet. Also like several other species, cats become transiently hypersensitive to the newly introduced dietary antigen prior to the establishment of tolerance. In contrast, there are a number of potentially important differences. In particular, there are significant differences between cats and dogs in the expression MHC class II molecules on gut epithelial cells. Similarly, it has been reported that cats have elevated numbers of intraepithelial lymphocytes (IEL) and that a proportion of these express surface IgM. It remains to be determined if these differences reflect the way in which the animals are maintained and if they may have greater biological significance.

cat / dog / mucosal / gut / immunology

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1. INTRODUCTION

The current state of our collective knowledge of mucosal immunology is a reflection of studies carried out on a number of species, most notably man, rodents, ruminants and pigs. In contrast it could be argued that in terms of contributing "at the cutting edge", studies on the mucosal immune systems of both cats and dogs have contributed relatively little. Whilst many of the studies in these companion animals have been aimed at confirming that which has already been reported in other species, there are a few notable exceptions. One such pioneering study was that of Cantor and Dumont. They were the first to highlight the important role of the liver in the development of oral tolerance. Using dogs they were able to demonstrate that portacaval shunting could abolish the tolerogenic effect of feeding the contact sensitising agent dinitro-chlorobenzene (DNCB) [13].

Whilst overall the strategies adopted by most species to control events at mucosal surfaces are essentially very similar, there are significant differences as to how this is accomplished. The most marked differences are found between ruminants and mono-gastric animals and not surprisingly both cats and dogs fit into the latter pattern. The aim of this review is to briefly summarise the similarities between what has been established in other species with that reported in cats and dogs and to focus upon areas where significant differences have been identified.

The mucosal surface of the gastrointestinal tract forms a major interface between any animal and the environment in which it lives. The gut mucosal environment is complicated by both the magnitude of challenge and the complex array of antigens that are presented. The immune system that is associated with the gastrointestinal tract is required to recognise these different groups of antigens and respond "appropriately". It must thus be able to respond actively to potential pathogens whilst at the same time not "over-reacting" to harmless components of the diet. In order to control such an extensive and diverse challenge, a complex battery of responses can be invoked. These include both innate and acquired mechanisms but it can be reasonably argued that the principal strategy adopted by both is one in which the response is directed toward preventing the antigen from interacting with epithelial cells and thereby closing a "potential gateway" into the body. The gut epithelial cells and their associated mucus layer along with peristalsis and the low stomach pH all contribute toward the barrier against the entry of harmful antigens.

The gastrointestinal tract is an extremely complex organ having multiple functions directed toward the digestion and absorption of nutrients, and the control of potentially harmful pathogens and commensal microflora. It is not surprising therefore that a well-developed mucosal immune system has evolved to protect it. The mucosal immune system can be divided into two major compartments: that consisting of the organised lymphoid structures (Peyer's patches, mesenteric lymph nodes, etc.) and that occurring in tissues specialised for other functions (the intestinal lamina propria). In the conventional model, the organised tissues are "inductive" sites, populated by naive cells: following priming the cells migrate via the mesenteric lymph node before homing to the diffuse, "effector" sites such as the intestinal lamina propria. Lymphoid aggregates are found throughout the intestine and it has been suggested that the numbers may reflect the bacterial load encountered in different areas of the feline large intestine [49]. The large numbers of aggregates in the anal canal and terminal rectum ("rectal tonsils") are thought to prevent ascending infection from the perianal area. In healthy animals, faecal material is present in the post-pelvic region only during defecation and the number of lymphoid aggregates is correspondingly low. Faeces are stored in the distal colon proximal to the pelvis, an area where there is a greater density of lymphoid aggregates. Moving away from the pelvis toward the ileocloic junction, the reduced

likelihood of faecal stasis may account for the gradual decline in the number of aggregates. Interestingly, a similar pattern is observed in the distribution of dividing (proliferating cell nuclear antigen positive - PCNA) epithelial cells with the numbers of PCNA positive cells increased with distance from the anus.

2. ENTEROCYTES IN MUCOSAL IMMUNITY

The innate immune defence system acts primarily at host barriers such as the gut mucosal surface and gut epithelial cells play a major role. Besides forming a highly specialised physical and functional barrier to dietary and microbial antigens they are able to recognise colonising micro organisms through expression of diverse receptor systems. These include glycan receptors that recognise fimbrial lectins found on many pathogenic and commensal strains of bacteria and viruses, Toll-like receptors (TLR) that recognise microbial molecular patterns and MHC class II molecules. There are significant differences between species in the expression of MHC class II molecules on gut epithelial cells. In the cat, there is no expression by villous or crypt enterocytes, but granular cytoplasmic staining of epithelial cells adjacent to Peyer's patches has occasionally been observed [56, 57]. Similar immunohistochemical studies on dog tissues have shown that whilst the duodenal epithelial cell expression of MHC class II molecules was faint and limited to the lower crypt region, jejunal and ileal enterocyte expression was stronger and present in both the crypt and villus areas. Enterocyte expression was of the greatest intensity in areas adjacent to the Peyer's patches [22]. Thus the pattern of expression in the dog would appear to have some similarity to that in rodents and man [6, 7, 38] whilst the paucity of epithelial MHC class II expression in the cat appears to be more similar to that reported in pigs [62]. In rodents there is constitutive expression by enterocytes at the villus tip and expression may also be induced in the crypt epithelium, secondary to a range of inflammatory disorders [8].

The unique location of gut enterocytes at the interface between the host and gut environment highlight their pivotal role in gut defence. It is then not surprising that there is a growing body of literature on their expression of various "accessory molecules" that may help facilitate this role. To date, relatively few markers have been studied in detail on cat and dog tissue. The expression of chemokine receptors has been investigated in the feline large intestine as well as the reproductive tract, using DNA probes specific for mRNA encoding for CCR3, CCR5 and CXCR4. CCR5 and CXCR4 receptor mRNA was expressed by epithelial cells (and some lamina propria cells) of the colon and rectum. Epithelial cell expression of chemokine receptor mRNA is reduced in intensity towards the base of crypts and the CXCR4 receptor was also demonstrated on a proportion of intraepithelial lymphocytes (IEL) [12]. TLR are an evolutionary conserved family of cell surface and cytosolic receptors which have an important role in microbial recognition. Recent studies in other species have highlighted their importance in innate immunity against pathogens and in immune homeostasis [1], and it is not surprising that related studies of their distribution have been reported for companion animals. TLR4 is a major receptor for bacterial endotoxin (LPS) and given the high bacterial load in the gut and upper airways it might be expected to play a pivotal role in mucosal defence. TLR4 mRNA has been shown to be expressed in the canine stomach and small intestine and the feline lung and small and large intestine [2]. Reassuringly immunohistochemical studies have also shown TLR4 in canine lung and small intestinal macrophages [58]. Using a similar argument, it could also be reasoned that TLR9, which recognises CpG-DNA (bacterial DNA), might also be expected to be widely expressed at mucosal sites. It is then perhaps surprising that TLR9 mRNA was not

detected in the lung or small and large intestine [33]. The observed differences in the tissue distribution of TLR's may be a reflection of their cellular localisation, for whilst TLR4 is expressed on the cell surface, TLR9 is confined to the endosomal compartment [35]. Equally these studies might also highlight that mucosal tissues contain a wide variety of cell types and the relative proportions of enterocytes, IEL, LPL could profoundly influence the outcome of analysis and thus it will be of importance to focus further on purified cell populations [35].

The main innate immune cell players are dendritic cells (DC), macrophages, neutrophils, γδ T cells and Natural Killer (NK) cells, which send out warning signals of pathogen presence as well as acting as effectors in eliminating pathogens. It should be emphasised that adaptive immunity (i.e. responses involving T and B cells with RAG-dependent rearrangement of antigen-receptor genes, and resulting in "memory") is crucially dependent on the innate immune system, both in its initiation (through DC interactions) and its effector phase (through involvement of myeloid cells and NK cells).

3. INDUCTIVE AND EFFECTOR SITES

There is a large body of evidence to show that, whilst Peyer's patches are the major site of induction of mucosal responses, the lamina propria and epithelial compartments are essentially involved in surveillance and the provision of help during the rapid responses to recall antigens. These effector responses include both active protective responses against potential pathogens and the prevention of damaging allergic responses to dietary and environmental antigens. Studies on the distribution of immune cell populations of both cats and dogs have focused primarily on the lamina propria and epithelial compartments with relatively few studies on Peyer's patches. The distribution of cells of feline Peyer's patches are reported to be similar to other species, with a greater number of B cells than T cells [34].

IEL are located in the epithelial compartment and are generally observed in close proximity to the basement membrane. There are considerable differences between species in the numbers of small intestinal IEL that have been reported, ranging from 12–20% epithelial cells in dogs [24, 52] to 51% epithelial cells in pigs [61]. Recent studies in the cat [56, 57] have shown that the number of IEL is much greater in this species. Feline IEL are more frequent in the villus than crypt (< 5% epithelial cells) epithelium and within the villus the number of IEL increases from the duodenum (about 50% epithelial cells) to the ileum (about 80% epithelial cells). Studies in the dog have also shown a greater number of IEL in the villus than crypt epithelium, but the numbers are similar in the duodenum and ileum [24]. Fewer studies have investigated the numbers of IEL in the large intestine, but Dobbins [17] reported 5% epithelial cells in man and Atkins and Schofield [3] 2% epithelial cells in dogs. The numbers of IEL in the feline large intestine are similar (about 4% epithelial cells) [48].

The phenotype of IEL has been investigated in both cats and dogs. In both species, $CD8^+$ IEL greatly outnumbered $CD4^+$ cells [24, 52, 57]. Whilst the numbers of αβ and γδ T cells in the canine villous epithelium are similar, the total number of $CD3^+$ IEL exceeds that of either population suggesting that as in other species, the IEL population in the dog is heterogeneous [25]. It would also appear from these studies that the number of the γδ IEL in the dog is larger than that reported in man, but comparable to that of the mouse [41]. Subtractive analysis of the $CD8^+$ feline IEL showed that almost half of the $CD3^+$ intraepithelial T cells were likely to be positive for CD8, which leaves a significant proportion of the IEL population ($CD4^-CD8^-$) unaccounted for. This was in agreement with studies on isolated gut cells which revealed high numbers

of $CD8\alpha^+$ lymphocytes (40%) in the epithelial compartment together with a significant population (44%) of $CD4^-CD8^-$ (double negative) lymphocytes [42].

Further recent reports of studies on isolated feline mucosal lymphocytes have confirmed that the vast majority of IEL are $CD5^+$ T cells [34]. Approximately 60% of these are $CD8^+$ with roughly half displaying $CD8\alpha\alpha$ homodimers. $CD4^+$ T cells make up no more than 10% of the total IEL pool.

In the canine small intestine lamina propria, T cells are distributed primarily in the upper villus with gradually decreasing numbers to the crypts. In contrast, the majority of B cells and plasma cells are present within the crypts with only a small number of cells within the villus [18, 24]. A similar pattern of B and T cell distribution has been described in the cat [56] and in other species such as the pig [55]. The reasons underlining the different distributions of B and T cells are unclear but it has been suggested that $CD4^+$ cells adjacent to the crypts are predominantly of Th2, whilst a greater proportion of the $CD4^+$ cells present in the upper villus may be of the Th1 phenotype. In both species, given the predominance of IgA bearing cells over those expressing IgG and IgM [24, 56], the colocalisation with secretory component expressing epithelial cells may also be significant. The polymeric immunoglobulin receptor (pIgR) which is required for the selective transport of IgA across epithelial cells to the gut lumen, is also largely restricted to the crypt region (for review see [39]).

The distribution of lamina propria $CD4^+$ and $CD8^+$ T cells is similar in cats and dogs, with significantly greater numbers of $CD4^+$ cells present [24, 56]. In the dog, the distribution of $CD5^+$ lymphocytes was similar to that of $CD3^+$ cells [24, 25], but given the precise function of CD5 the significance of this finding remains speculative. These authors have also reported [24] that the majority of LPL (and IEL) stained with a monoclonal antibody which reacts with a CD45 isoform. Although the precise lineage of these $CD45R^+$ cells was not determined, their very widespread distribution led the authors to conclude that a significant proportion of these heterogeneous cells might include a population of naïve T cells. If this was to be confirmed, then it means that the dog differs from all other species which have consistently shown that lamina propria cells are of an activated or memory phenotype [28]. Recently reported studies on isolated feline mucosal lymphocytes have shown that the percentage of $CD4^+$ $CD25^+$ T cells is greater in both IEL and LPL from random source compared with specific pathogen free cats [34]. Whilst this finding supports the hypothesis that antigen exposure can impact upon the numbers of activated or memory cells in the intestine, the lack of any differences with other markers [34] suggests the need for further study.

Reflecting their pivotal role as an inductive site for mucosal immune responses, Peyer's patches display the greatest expression of MHC class II antigens, with lower levels of expression in the epithelial and lamina propria compartments [34]. Within the feline lamina propria, MHC class II molecules are expressed predominantly by cells with macrophage or dendritic cell morphology [56]. The number of positive cells was greater in the villus than crypt areas. A similar pattern of staining has been described for the dog with no difference between anatomical regions of the small intestine. In both species, further analysis of the macrophage populations have been performed using the monoclonal antibody MAC 387. This antibody detects the myelomonocytic L1 antigen in human tissues [9] and although the true specificity of this marker has not been confirmed for feline or canine tissue, it has been reported to recognise cells with the morphological characteristics of macrophages/monocytes in cats and granulocytes and a subset of macrophages in dogs. In cats, MAC 387+ cells are found evenly distributed between the villus and crypt areas, with a greater number of cells in the ileum than the other regions of the

small intestine [56, 57]. In the dog, it was similarly found that the greatest density of cells was found in the ileum, but in this species they also have a greater predilection for crypt over villus areas [24]. The distribution of mast cells have also been described for the canine small intestine, being mainly found in the subepithelial lamina propria, with small numbers present within the muscle layers [24, 25].

4. MUCOSAL IMMUNOGLOBULINS AND PLASMA CELLS

As with most other mammals, IgA in the cat is the predominant immunoglobulin in mucosal secretions [54]. It is found in large amounts in saliva, tears, respiratory and intestinal secretions, milk and bile [44]. In the small intestinal lamina propria IgA-producing cells predominate, accounting for 40 to 80% of the total number of plasma cells. In contrast, IgG producing cells are more numerous in colonic tissues with smaller numbers of IgA and IgM producing cells [36]. More recent studies have been sought to precisely map the distribution of plasma cells within the villus crypt unit. As with other species where similar studies have been completed, IgA plasma cells increased from the villus to the base of the crypts. Within the crypts there is a trend for the numbers of IgA cells to increase from the duodenum to the ileum [56]. It is tempting to suggest that this pattern of distribution might be a reflection of the increased bacterial load in the ileum [45], but if this was the case it would be difficult to reconcile with the findings in the dog and pig where the total number of plasma cells is the greatest in the duodenum [10, 24, 32, 60].

An unusual distribution of IgM positive cells has recently been noted in cats. Whilst overall the numbers were greatest in the jejunal lamina propria, a small number of IEL expressing cytoplasmic IgM were also reproducibly observed [56]. IgM positive IEL have not been recognised in other species and their biological significance in feline mucosal immunity has yet to be determined.

The origin of the immunoglobulins that appear on mucosal surfaces is most easily addressed with secretions such as saliva and tears. The general lack of correlation between the relative concentrations of IgG, IgA and IgM serum, saliva and tears serves to highlight that serum immunoglobulin concentrations are poor indicators of what appearing in fluids that bathe these mucosal surfaces. In canine tears and saliva, albumin concentrations correlate with IgG but not with IgM or IgA, whilst IgM and IgA concentrations are correlated with each other [23]. This would suggest that IgG like albumin appears in these secretions as a result of transudation from serum. In contrast IgA and IgM are likely to appear as a result of local production (e.g. in the lachrymal gland) and or selective active transport. Evidence of local production has been sought using a small intestinal gut explant culture system [26]. There was a gradual increase over 24 h in IgA appearing in culture supernatants whilst the concentrations of IgM and IgG did not change. Blocking studies with the protein synthesis inhibitor cyclohexamide showed a dose dependant reduction in IgA appearing in culture supernatants, providing strong evidence for local production in the intestinal lamina propria.

The origin of immunoglobulins in feline saliva has also been addressed [29]. These authors showed that IgA is the predominant immunoglobulin secreted by the major feline salivary glands, reflecting the greater number of IgA bearing plasma cells at this site [64]. The level of immunoglobulins detected in saliva following "stimulation" with lemon juice was lower than in unstimulated samples and the relative proportion of each immunoglobulin class and albumin differed. The latter finding would suggest that stimulated saliva cannot simply be considered to be a diluted form of unstimulated saliva and highlights that whole saliva is a complex fluid comprising the products of a number of different sources. Studies in cats

with chronic gingivostomatitis would serve to emphasise this point. Cats with chronic gingivostomatitis have significantly higher salivary concentrations of IgG, IgM and albumin, and higher serum concentrations of IgG, IgM and IgA, but significantly lower levels of salivary IgA than healthy cats [31]. Prior to treatment, the levels of oral inflammation were not correlated with serum or salivary immunoglobulins, however following treatment the improvement in the "stomatitis index" was significantly correlated with changes in the cat's salivary IgM and IgA concentrations. A similar reduction in salivary IgA concentrations have been reported during infections in man and are thought to be the result of a combination of a reduction in IgA synthesis and a reduction in salivary flow [51].

5. MUCOSAL CYTOKINES AND THE RESPONSE TO MUCOSAL INFECTION

The vast majority of studies on the distribution and role of cytokines at mucosal surfaces in both cats and dogs have been restricted to assaying cytokine mRNA by PCR based techniques. This is a reflection of the relative paucity of antibodies to companion animal cytokines and therefore any results obtained by these methods carry the caveat that mRNA transcripts may not necessarily correlate with protein expression. Using a semi-quantitative RT-PCR, it has been shown that the cytokine profile of the "healthy" feline oral mucosa is dominated by IL-2, IL-10, IL-12 (p35 and p40) and IFN-γ [30]. These authors also showed that in cats with chronic gingivostomatitis, there is upregulation of these cytokines and the expression of IL-4 and IL-6 within the oral lesions [30]. Bovine lactoferrin has a variety of biological properties and it has been reported that oral administration can ameliorate oral inflammation in FIV infected cats with intractable stomatitis [43]. The mechanisms underlying the observed clinical improvement have not been fully elucidated but in cats it has been reported that, bovine lactoferrin can also reduce IFN-γ production by concanavalin A stimulated peripheral blood mononuclear cells [37]. Interestingly, related studies of intestinal inflammation in rats have also shown a clinical improvement following oral administration of bovine lactoferrin and this was associated with an enhanced production of IL-4 and IL-10 and a reduction in TNF-α, IL-1β and IL-6 [53].

The majority of studies of feline mucosal cytokines have focused upon changes detected following infection. In a recent study of cats rectally infected with FIV cytokine, mRNA levels for IFNγ, TNFα , IL-4, IL-2, IL-6, IL-10, and IL-12 have been assayed in colonic lymph node CD4 and CD8 cells [4]. Interestingly, the initial phase of the response (when viral replication was the greatest) was dominated by IL-10 production by both $CD4^+$ and $CD8^+$ cells. Subsequently (between weeks 4 to 10 post-infection) FIV levels in tissues decreased and IFNγ production by $CD8^+$ T cells increased to restore the IL-10/IFNγ ratio to pre-infection control levels. These authors [4] suggested that the temporal associations of viral replication and tissue cytokine balance might be critical in controlling local lentiviral infection.

The effect of bacterial infection on local cytokine production has also been investigated. *Helicobacter pylori* infection in cats is associated with lymphofollicular gastritis, with sub-mucosal lymphoid follicles distributed most frequently in the antrum of the stomach. The gastric lymphoid follicles consist mainly of IgM^+ B cells surrounded by clusters of $CD4^+$ and $CD8^+$ T cells [21]. More recent studies [47] have sought to characterise the cellular and cytokine changes in gastric tissues that occur during the early stage of infection in cats. The initial mucosal response was associated with an increase in the levels of IFNγ, IL1α, IL-1β and IL-8 mRNA with secondary lymphoid follicles infiltrated with BLA.36 positive cells (progenitor B cells), CD79α positive

cells (reactive B cells), and CD3 positive T cells. Interestingly, the follicles were negative for B220.

A recent study has sought to unravel the relationships between cellular and cytokine changes with *Helicobacter* spp. infection in a group of dogs presenting clinical gastritis. The authors reported that the mucosal pathology could be related to cytokine mRNA expression (neutrophils to IL-8 and IFN-γ, macrophages and lymphocytes to IFN-γ, and gastric fibrosis to IL-1β). Approximately 75% of the cases were found to be infected with *Helicobacter* spp. and this was associated with elevated levels of TGFβ and gastric fibrosis [59]. To date, the majority of studies on mucosal cytokines in both cats and dogs have adopted the semi-quantitative RT-PCR for the analysis of gut tissue. The semi-quantitative nature of this test severely limits the interpretation of the results obtained and more recent studies have sought to overcome this limitation with the use of real-time RT-PCR. These assays provide more accurate and sensitive methods of quantifying mRNA transcripts. For dog cytokines, real-time assays have been developed for mRNA encoding IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, IFNγ, TNFα, and TGFβ [40]. These assays have been applied to "normal" canine duodenal mucosa where transcripts IL-18, TGFβ and TNFα were found to be the most abundant, with IL-10 and IFNγ present at levels approximately 10-fold less [40]. German shepherd dogs are predisposed to enteropathies such as inflammatory bowel disease and small intestinal bacterial overgrowth. Earlier studies from the same laboratory had used a semi-quantitative PCR on these dogs to investigate a possible relationship between gut pathology and cytokine changes. IL-2, IL-5 IL-12p40, TNFα and TGFβ_1 were all shown to be elevated in German shepherd dogs with small intestinal enteropathies [27]. In these dogs, treatment with antibiotic (oxytetracycline or tylosin) resulted in reduced TNFα and TGFβ_1 templates, supporting the conclusion of an immune mediated gut pathology.

6. CELL TRAFFICKING AND HOMING

There is a large body of evidence to support the observation that mucosal cells are distinct from those found at non-mucosal sites. Such evidence includes those based on phenotypic analysis, migration and trafficking studies as well as functional properties. In order to mount an effective mucosal immune response, cells are required to traffic between inductive (Peyer's patch) and effector sites (lamina propria and epithelium). This migratory pathway requires the interaction between the ligand α4β7 (expressed by "mucosal lymphocytes") and the mucosal cell addressin molecule, MAdCAM-1, which is expressed on the vascular endothelium in mucosal tissues. Studies of the distribution of MAdCAM-1 in canine tissue have confirmed that it is expression is restricted to endothelial cells in GALT, including Peyer's patches, mesenteric lymph node, intestinal mucosa, submucosa and muscularis [25]. A pattern of expression is similar to that reported for other species. Whilst the expression α4β7 has been associated with the homing of cells to the lamina propria, another member of the β7 subfamily of integrins has been implicated in the localisation of IEL. Studies in other species have found αEβ7 expressed on the overwhelming majority of IEL, but with a smaller number of LPL (about 50%) and very few peripheral blood cells positive for this marker [14]. The feline αE integrin has been cloned and sequenced, showing approximately 70% homology with human and rodent counterparts at the nucleotide level [63]. The tissue distribution and biochemical properties were also found to be largely similar to those reported for other species, strongly indicating that in the cat it may also be playing a role in orchestrating the interactions between IEL and E-cadherin positive epithelial cells [63].

7. INDUCTION OF MUCOSAL IMMUNE RESPONSES

Two of the key reasons that underlie the need for a better understanding of mechanisms that operate at mucosal surfaces, are an ability to control infections through the development of mucosal vaccines and the protection from allergic reactions through the development of oral tolerance. There is a large body of data to show that immune responses that are protective at mucosal surfaces are most effectively stimulated by local application of the antigen. The expression of active immune responses against antigens presented to the mucosa is frequently disadvantageous for an individual organism. The induction of responses, proliferation of appropriate cell types and synthesis and secretion of appropriate effector molecules require diversion of energy and resources from other systems. The effector mechanisms of immune responses frequently result in tissue inflammation and damage, independent of that generated by the pathogen. Presumably, the temporary disadvantage of expression of immune responses outweighs the long-term disadvantage of having to live, or die, with the pathogen. Since the pathogenicity of micro-organisms varies from severe (e.g. *Vibrio cholerae*) to low or absent (true commensal flora, food), this also requires an ability to modulate immune responses dependent on the perceived threat, independent of the antigenic load. That is, the magnitude and type of response should be dependent on the "quality" of the antigen, not solely on the quantity. In the case of most food antigens in normal individuals, this would, ideally, involve complete absence of immune responses or "immunological tolerance". Studies in both cats and dogs suggest that such complications as those described above are equally applicable to both cats and dogs.

Oral tolerance is a specific acquired mechanism whereby prior feeding reduces an individual's ability to respond to subsequent presentation of that antigen [50]. The induction of oral tolerance has been very extensively studied in rodents and a number of regulatory processes have been characterised. Although fewer studies have been performed, it is clear that tolerance can be induced in both cats[1] and dogs [16]. The studies in rodents have identified a number of factors (e.g. age, genetic, dietary change, microbial flora, weaning) that can abrogate or delay the induction of mucosal tolerance. It is to be expected that a similar range of factors may also play a role in determining the outcome of feeding novel dietary proteins in both cats and dogs. If this is so, then the differences in the induction of tolerance in these species are likely to underlie a number of gut pathologies including inflammatory bowel disease.

The use of mucosal vaccines in cats has an impressively long history. A live cold-adapted feline herpesvirus type 1 (FHV-1) intranasal vaccine that mimics the "natural method of infection" was first described in 1976 [46]. The vaccine provided a rapid inset of protection with partial protection from challenge after two days and complete protection by day 4 [15]. Whilst these studies highlight the potential for live attenuated mucosal vaccines, the lack of availability of similarly attenuated strains for other viral infections, and concerns over safety have restricted their wide spread application. More recent studies with feline immunodeficiency virus (FIV) serve to highlight the difficulties associated with generating protective mucosal responses. FIV is a natural pathogen of cats, which although it is considered to be normally transmitted by biting, can be experimentally infected by both rectal and vaginal routes [5, 11]. Experimental studies have demonstrated that vaccination regimes that provide protection from intraperitoneal challenge fail to protect from rectal or vaginal challenge [19]. Studies in other species have identified a range of experimental approaches to overcome this, but generally they have failed to elicit protection

[1] Waly N., Ph.D. thesis, University of Bristol, United Kingdom.

when applied to FIV infection of cats. For example whilst both cholera toxin and lipo thioester enhanced antibody and cellular responses to FIV peptides, they failed to protect against rectal challenge [19]. The results from two other studies may provide some scope for optimism and possible approaches for further development. The first of these, targeted lymph node immunisation, is based upon injecting antigen directly into the local lymph node that is involved in drainage from a specific mucosal site. The medial iliac lymph nodes are the principal site of drainage and migration of cells from the genital and rectal mucosa and immunisation into the region of these nodes has been shown to provide protection from rectal challenge with both cell-free and cell associated FIV [20]. Unfortunately, the technical difficulties associated with this route of immunisation would make it an unlikely candidate for widespread application. An alternative successful approach has involved *Listeria monocytogenes* as a vaccine vector. Three months after oral immunisation with a recombinant *L. monocytogenes* that expresses the FIV Gag and delivers an FIV Env-expressing DNA, cats were vaginally challenged with a molecular clone of FIV. Whilst virus was isolated from four of the five immunised cats, further analysis indicated that viral load was reduced [48]. Taken together, these studies highlight the potential of the mucosal route of immunisation and support the widely held dogma that live vaccines are more effective than killed or subunit vaccines. Given the continuing concerns over the use of live vaccines, either as attenuated strains or recombinant vectors, the need for an alternative approach involving the use of a killed or subunit vaccine given together with a novel mucosal adjuvant remains.

In recent years there has been significant progress in charactering the mucosal immune systems of both cats and dogs. Despite this, resolution of the key challenges, to prevent harmful allergic reactions, and to control mucosal infections, remains some way off. The key to both, being dependent upon a greater understanding of the cellular and molecular mechanisms involved in the induction and regulation of responses at mucosal surfaces.

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