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Understanding gastrointestinal inflammation — implications for therapy

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It is now apparent that the immune system at the intestinal mucosal surface (eg, gut-associated lymphoid tissue-[GALT]) has distinct structural and functional features. Indeed, the GALT is a most complex organ which intimately interfaces between the environment and the host organism. These functions include tolerance to orally administered antigens, local protective immune responses at the B and T cell level, and systemic and mucosal dissemination of stimulated B and T lymphocytes. In addition, animal models of intestinal inflammation have shown that disturbances in immune regulation lead to mucosal inflammation. A breakdown in mucosal tolerance is likely to be a key feature in the development of chronic gastrointestinal inflammation, such as seen with inflammatory bowel disease (Duchmann et al 1995).

GALT and the mucosal barrier

The GALT is compartmentalised into both afferent and efferent sites. The afferent arm (where antigen exposure leads to a primary immune response) consists of mesenteric lymph nodes, lymphoid nodules, and Peyer's patches; while effector limb effector functions are performed by the lamina propria (LP) and intestinal epithelium. Both arms of GALT are coupled by migration of stimulated lymphocytes from afferent sites into the systemic circulation, where selective homing to effector sites is facilitated by high endothelial venules expressing several adhesion molecules. The gastrointestinal LP consists of multiple cellular elements including plasma cells, T cells, and dendritic cells. B cells predominate in the intestinal LP, with the majority being of the IgA isotype (Willard & Leid, Hart 1979). Plasma cells are distributed non-uniformly along the small intestine and the density of IgA+ plasma cells decreases from the duodenum to the ileum. CD3+, CD4+, and CD8+ T cells are all found within the LP with most residing near the luminal surface. CD4+ T cells predominate in the canine LP (German et al 1991) while CD8+ T cells are most numerous in the villus epithelia, with a significant population of these intraepithelial lymphocytes (IELs) expressing T cell receptor γδ (TCRγδ) (Sonea et al 2000).

CD4+ T cells are a major cytokine producing cell that provide help for immune effector cell populations (Garden). Recent evidence has shown that different CD4+ T cell populations have different patterns of cytokine secretion which function homeostatically to regulate (balance) mucosal immune responses. A T helper 1 (Th1) population, which supports cell-mediated immunity, is characterised by secretion of IL-2, IFN-γ, and TNF-α while a Th2 population favours antibody production and the secretion of IL-4, IL-5, and IL-10 (Mosmann & Coffman 1989). Both IL-10 and TGF-β are important down regulatory cytokines that are involved in the maintenance of oral tolerance, and facilitate the

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production of IgA by plasma cells (Groux et al 1997). Most immune responses are not solely regulated by CD4+ T cells but are instead governed by the local environmental cytokine milieu.

The mucosal barrier provides a formidable challenge that limits antigenic exposure to GALT. Principal components include gastric acid, mucus, digestive enzymes, peristalsis, resident microflora, luminal epithelia, and sIgA. Other innate factors (lysozyme, lactoferrin, defensins, complement, and more) produced by cells within the intestines contribute to the innate antimicrobial activity of the gut. The GALT and mucosal barrier are closely interfaced to prevent untoward immune responses which cause chronic gastrointestinal inflammation.

Chronic mucosal inflammation — pathogenesis

The potential responses of GALT to an intraluminal antigen may include exclusion, tolerance, or mucosal inflammation. Animal models have shed new light on mechanisms of mucosal immune dysfunction suggesting that the fundamental defect ca using chronic intestinal inflammation is a breakdown (loss) of mucosal tolerance. Factors contributing to this defect include abnormalities in the mucosal barrier, the resident bacterial flora, and disruption of GALT. Barrier disruption-mediated inflammation has recently been demonstrated in mutant mice having variable expression of N-cadherin (an intercellular adhesion molecule) in small intestinal epithelia (Hermiston & Gordon 1995). Expression of this mutation results in loss of N-cadherin in affected cells accompanied by epithelial disruption and localized intestinal inflammation. Compelling evidence now implicates the resident bacterial flora as an essential cofactor in driving mucosal inflammation. However, members of the microflora have differing abilities to mediate gut inflammation. *Bacteroides vulgatus*, members of the enterobacteriacae, and *Helicobacter* spp (eg, *H hepaticus*) appear to augment intestinal inflammation; while, *Lactobacilli* spp are less pathogenic (Madsen et al 1999). Also, inflammation is prevented in animal models of IBD when they are maintained in germ-free conditions as compared to conventional facilities. Lastly, mice having targeted deletions of genes encoding for IL-2, IL-10, or TGF-β readily develop intestinal inflammation suggesting a prominent role for T cells (such as CD4+) in disease pathogenesis. Summarising, most gastrointestinal inflammation probably occurs due to abrogated mucosal tolerance caused by these three principal mechanisms.

Mechanisms of mucosal inflammation

Mucosal inflammation is the vascular and cellular response of intestinal tissue to injury. This initially is a protective mechanism in which immunoglobulins, complement, and other serum constituents are concentrated at the sites of tissue damage. Key features of this response include vasodilatation, increased vascular permeability, chemotaxis, and increased cellular functions (eg, phagocytosis etc.). If the inflammatory response fails to eliminate the cause of injury, then chronic inflammation ensues. Consequences of mucosal inflammation are numerous and include elimination of microorganisms and foreign antigen, tissue healing, tissue damage, altered motility, increased intestinal permeability, and systemic consequences. A number of chronic enteropathies are known to exist which may have an underlying immune-mediated aetiology for tissue injury, including dietary sensitivity, small intestinal bacterial overgrowth, and inflammatory bowel disease (IBD). Special emphasis will be placed on canine IBD as one model for chronic mucosal inflammation that has recently been investigated.

Canine inflammatory bowel disease — immunodiagnostic perspectives

Canine IBD is a chronic gastrointestinal disorder of unknown cause and ill-defined pathogenesis. As in human IBD, this disorder results from complex interactions between host susceptibility, mucosal immunity, and environmental factors (eg, dietary antigens, resident microflora). Recent immunological and molecular studies have suggested a role for mucosal immune dysfunction in the pathogenesis of canine IBD. Rectal dialysates from dogs with active lymphocytic-plasmacytic colitis contain elevated concentrations of nitrite (a stable metabolite of nitric oxide) and IgG (Gunawardana et al 1997). Similarly, other studies have shown increased mucosal concentrations of iNOS (inducible nitric oxide synthase) in endoscopic biopsies from dogs with small and large intestinal IBD (Jergens et al 1998).

Immunohistochemical techniques have also shown increased lamina proprial T cells (primarily expressing αβTCR and CD4+ in small intestinal tissues and CD8+ in colonic tissues) and plasma cells (IgG plasma cells with small bowel IBD and IgG and IgA plasma cells in colonic IBD) from diseased dogs (German et al 2001, Jergens et al 1999). Additionally, flow cytometric analysis of endoscopic specimens has revealed that IBD dogs contain fewer γδTCR cells in the IEL compartment, suggesting a possible defect in mucosal immune regulation (Jergens et al 2001).

Using RT-PCR techniques, other workers have documented up-regulated mucosal cytokine mRNA expression for IFN-γ, IL-12, TNF-α, IL-5, and TGF-β in dogs with small intestinal IBD (German et al 2000). Lastly, serological markers (eg, C reactive protein and haptoglobin) have shown good correlation to histological and clinical indices of disease activity, suggesting a possible role for these substances in measuring intestinal inflammation (Jergens). These accumulated observations offer persuasive evidence for mucosal immune system disturbances in canine IBD.

Gastrointestinal inflammation of canine IBD — immunotherapeutic implications

Effective modulation of mucosal inflammation is presently achieved with dietary modification and immunomodulating agents (eg, corticosteroids, sulfasalazine, azathioprine) which act non-specifically to inhibit or decrease the formation of inflammatory mediators or to block their specific receptors. Immunosuppressants are routinely used in animals with IBD as both induction agents and to maintain remission. Use of these agents is largely empirical as the optimal drug, combination of drugs, and duration of therapy has not been established. However, alterations in serum C reactive protein and haptoglobin concentrations appear to correlate with successful immunomodulatory therapy in canine IBD.

The contribution of diet to the development of intestinal inflammation, although less clear in human IBD, is better appreciated in animals. Studies of canine and feline IBD have strongly implicated dietary antigens as contributing to gastrointestinal signs, since signs resolve when novel diets are fed and resolution of signs, followed by recurrence of signs, is observed with re-exposure of the incriminating dietary antigen. One other explanation for clinical efficacy might be that dietary factors alter luminal microbe populations to reduce intestinal inflammation.

Manipulation of the resident bacterial flora through the use of antibiotics or dietary substances (prebiotics) may reduce intestinal inflammation of IBD. Numerous anecdotal reports and select studies attest to the efficacy of metronidazole for therapy of IBD in humans and animals. Only subtle modification of the bacterial flora has been reported with the use of fructooligosaccharides in animals. Probiotics are live microbial food ingredients that alter the enteric flora and have a favourable effect on health. A variety of organisms have been utilized in animal models of IBD including lactobacilli, bifidobacteria, and other nonpathogenic bacterial strains with encouraging results (Shanahan 2001). However, rigorous evaluation of probiotic therapy in humans and animals with IBD has not been performed.

Modulation of the enteric micro-environment has been recently shown to reduce pro-inflammatory mucosal cytokines (thereby attenuating intestinal inflammation) in humans with Crohn's disease (Shanahan 2001); and, similar beneficial effects are likely to be observed in dogs and cats with IBD. However, the optimal therapeutic manipulation — administration of immunomodulating drugs, dietary manipulation, and/or probiotic therapy — remains to be determined.

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