

## Chronic Intestinal Inflammation and Intestinal Disease in Dogs

A.J. German, E.J. Hall, and M.J. Day

Normal individuals maintain tolerance to the endogenous bacterial flora residing within their alimentary tract, a phenomenon mediated by the gastrointestinal lymphoid tissue. Loss of this tolerance is a key factor in the development of chronic intestinal inflammation. Manifestations of such uncontrolled inflammation in humans include inflammatory bowel disease and celiac disease. Dogs may similarly be affected, and although the etiopathogenesis is likely similar, the lesions differ. This review includes discussion of the factors involved in breakdown of mucosal tolerance, the immunologic basis of canine enteropathies, and the use of novel immunotherapies for these diseases.

**Key words:** Enteropathy; Gluten sensitivity; Inflammatory bowel disease; Mucosa; Small intestinal bacterial overgrowth.

The intestinal immune system is constantly exposed to a vast array of antigens, including those derived from food, components of the endogenous microbial flora, and pathogenic organisms. Important decisions must be made about the nature of the antigenic stimulus so that protective responses are mounted to pathogens but tolerance to harmless substances is preserved. If this delicate balance is interrupted, a state of chronic uncontrolled inflammation may ensue.

### Breakdown of Mucosal Tolerance Is Key to the Development of Chronic Intestinal Inflammation

Much of our knowledge of gastrointestinal inflammatory processes has arisen from the study of laboratory animal models (Table 1).<sup>1</sup> In such models a variety of spontaneously arising or induced disruptions of the mucosal immune system can lead to chronic inflammation, the end result of which is pathologically similar among models. These experimental studies suggest that a disruption in 1 of 3 critical areas, ie, the mucosal barrier (maintained by the epithelium), an appropriately functioning mucosal immune system, and the endogenous microflora, results in chronic inflammation (Fig 1).

The requirement for a protective barrier is clearly demonstrated by the N-cadherin–dominant negative chimaeric mouse.<sup>2</sup> These mice have variable expression of an N-cadherin mutant in the small intestinal epithelium, and such a mutation results in loss of E-cadherin (a molecule responsible for cell adhesion and maintenance of epithelial integrity) from the epithelial cells. In areas where the mutant gene is expressed, epithelial disruption occurs and there is

localized intestinal inflammation, whereas the areas with normal expression are free from inflammation.

Mucosal immune system dysfunction can also lead to the development of intestinal inflammation, and most models involve the targeted disruption of genes encoding immunologically active molecules such as cytokines. Intestinal inflammation develops in mice carrying disruptions in genes encoding interleukin (IL) 2, IL-10, or transforming growth factor (TGF)  $\beta$ .<sup>1,3–5</sup> Because these cytokines are mostly T cell derived, most of these models also demonstrate a critical role for T cells (and particularly the CD4<sup>+</sup> subset) in disease pathogenesis. The importance of CD4<sup>+</sup> T-cell subsets and cytokines is also demonstrated by the CD4<sup>+</sup> T cell transplanted severe combined immunodeficient mouse.<sup>6–14</sup>

A final factor that is of critical importance in the development of intestinal inflammation is the presence of an endogenous flora. In this regard, chronic intestinal inflammation generally does not develop when the mice in these model systems are reared in a germ-free environment.<sup>1</sup> This finding indicates that the development of an aberrant immune response to components of the endogenous bacterial flora is critical to the pathogenesis of chronic intestinal inflammation and inflammatory bowel disease (IBD). This hypothesis has been supported in recent studies by Duchmann and coworkers.<sup>15,16</sup> Normal humans and mice are tolerant of their endogenous intestinal flora but not of the flora of other individuals.<sup>15,16</sup> However, in humans with IBD or mice with chronic intestinal inflammation induced by trinitrobenzene sulphonic acid, this tolerance to autologous flora was lost. Regardless of the inciting cause for chronic intestinal inflammation, the presence of endogenous flora causes augmentation of the inflammatory process and ultimately leads to similar pathologic changes within the mucosa.

### Canine IBD

The canine idiopathic IBDs are a group of disorders characterized by persistent or recurrent clinical signs of gastrointestinal disease of undetermined cause associated with histologic evidence of inflammation in the small or large intestinal mucosa.<sup>17</sup> These diseases are classified according to the predominant type of inflammatory cell present and the area of intestine affected. Given that the diagnosis is histologic, the term IBD likely encompasses a range of disorders with as yet undiscovered etiologies. A variety of forms have been described, the most common of which is

---

From the Departments of Clinical Veterinary Science (German, Hall) and Pathology and Microbiology (German, Day), University of Bristol, Bristol, UK.

Reprint requests: Alex J. German, BVSc, PhD, CertSAM MRCVS, Department of Clinical Veterinary Science, University of Bristol, Langford House, Bristol BS40 5DU, UK; e-mail: a.j.german@bris.ac.uk.

Submitted November 16, 2001; Revised February 14, 2002; Accepted March 28, 2002.

Copyright © 2003 by the American College of Veterinary Internal Medicine

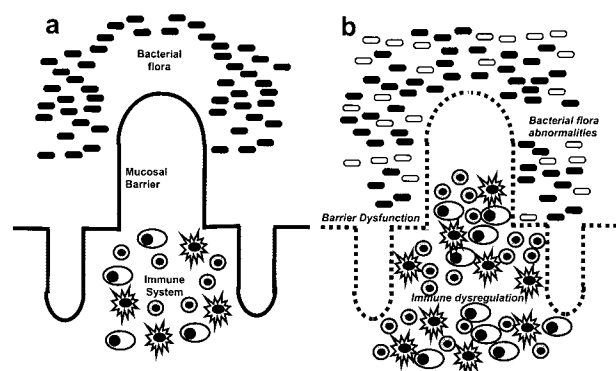
0891-6640/03/1701-0000/\$3.00/0

**Table 1.** Experimental animal models of chronic intestinal inflammation.

Category	Model
Spontaneous	Ulcerative colitis (cotton-top tamarins) C3H/HeJBir mouse
Induced	
Chemical	Acetic acid enema Immune complex/formalin enema TNBS/ethanol enema Indomethacin
Microbial products	Dextran sulphate sodium Carrageenan Lymphogranuloma venereum Peptidoglycan-polysaccharide
Genetically manipulated	
Cytokine perturbations	IL-2 deficiency IL-2R deficiency IL-10 deficiency TGF $\beta$ deficiency IL-7 transgenic
T cell or MHC perturbations	TCR $\alpha$ -chain deficiency HLA-B27/ $\beta$ 2m transgenic Cyclosporin A colitis
Epithelial perturbations	G $\alpha$ i2 deficiency N-cadherin dominant negative transgenic chimera Mdr-1 deficiency
Leukocyte transfer to immunodeficient rodents	CD45RB <sup>hi</sup> CD4 T cell into SCID mouse CD45RB <sup>hi</sup> CD4 T cell into RAG-deficient rats T cell transfer into SCID mouse Bone marrow transfer into Tg $\epsilon$ 26 mice Transfer of cells from mice infected with murine retrovirus

TNBS, trinitrobenzene sulphonic acid; IL, interleukin, TGF, transforming growth factor; MHC, major histocompatibility complex; TCR, T cell receptor; HLA, human leukocyte antigen; Mdr-1, multi-drug resistance gene 1; SCID, severe combined immunodeficiency; RAG, recombinase activating gene.

lymphocytic-plasmacytic enteritis.<sup>17</sup> A number of forms of IBD unique to certain breeds of dog also have been described, including histiocytic ulcerative colitis in Boxer dogs,<sup>18</sup> immunoproliferative enteropathy of Basenjis,<sup>19–23</sup> a diarrheal syndrome in Lundehunds,<sup>24</sup> and protein-losing en-

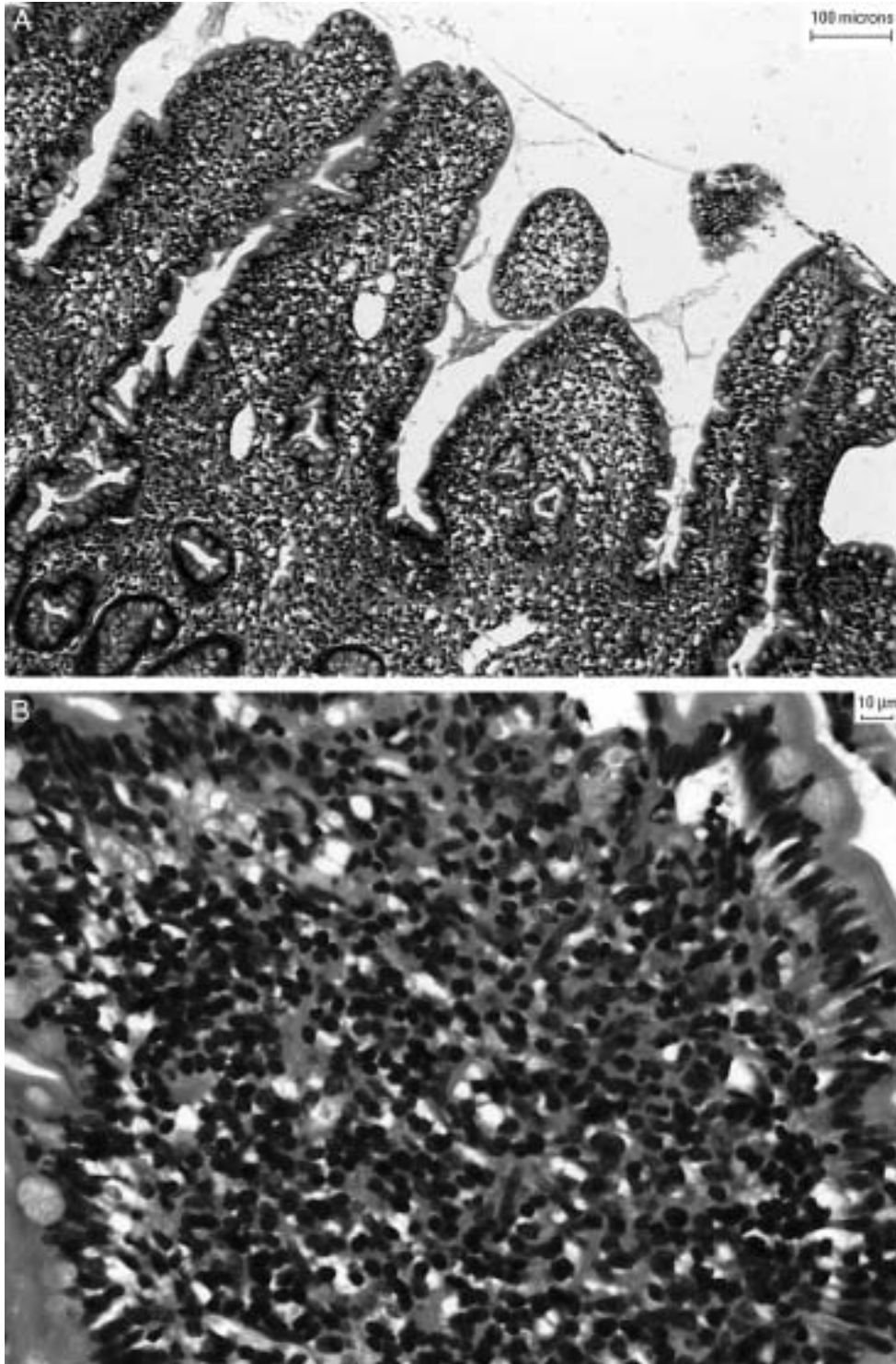


**Fig 1.** Aberrations giving rise to the development of chronic intestinal inflammation. (a) Normal small intestinal mucosa. The mucosal barrier is intact and separates the endogenous bacterial flora from the mucosal immune system. (b) Chronic intestinal inflammation can develop from disruption of the mucosal barrier, dysregulation of the mucosal immune response, and alteration in the bacterial flora.

teropathy and associated protein-losing nephropathy in Soft Coated Wheaten Terriers.<sup>25–28</sup>

Clinical and mechanistic similarities between IBD in dogs and IBD in humans exist; however, the histopathologic changes and area of the intestine predominantly affected are different. The 2 predominant types of canine IBD are lymphocytic-plasmacytic (Fig 2) and eosinophilic enteritis (Fig 3), characterized by infiltration of the respective cell type into the mucosa. Virtually any area of the gastrointestinal tract can be affected, but lesions most commonly involve the small intestine. Conversely, the 2 main entities of human IBD are Crohn's disease and ulcerative colitis.<sup>29</sup> Crohn's disease is characterized by focal and segmental granulomatous lesions of any portion of the gastrointestinal tract, particularly the ileum, with the rectum most commonly spared. Ulcerative colitis is a diffuse superficial disease, usually characterized grossly by ulceration and histopathologically by infiltration of inflammatory cells (neutrophils, lymphocytes, and plasma cells) into the rectal, colonic, and occasionally ileal mucosa.<sup>30</sup>

The pathogenesis in both species is likely immune mediated with immunologic, environmental, and genetic factors contributing to expression of disease.<sup>31–33</sup> Environmental factors include microbial antigens and dietary antigens. No specific infectious agents have been shown to cause

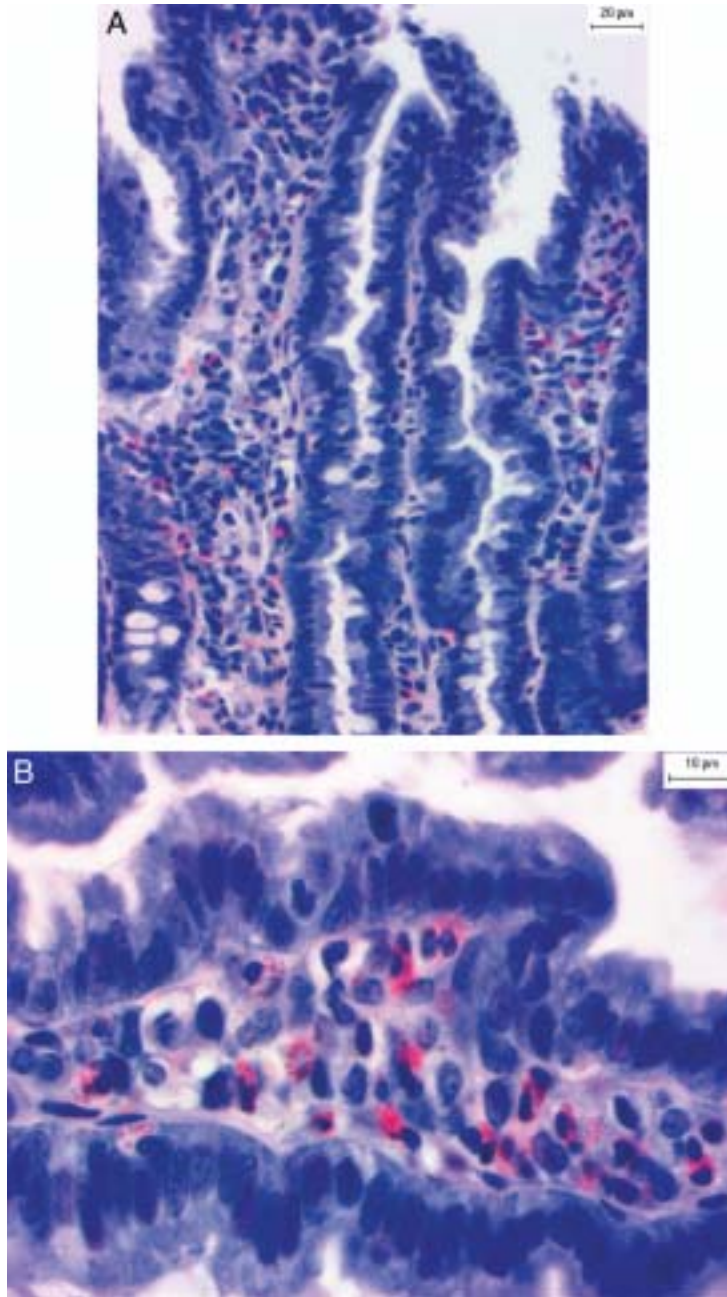


**Fig 2.** Duodenal mucosa from a dog with lymphocytic-plasmacytic enteritis. There is evidence of architectural abnormalities in conjunction with alterations in mucosal immune cell populations, which are predominantly lymphocytes and plasma cells. Hematoxylin and eosin.

canine IBD, although antigens derived from the endogenous microflora are likely to play an important role in disease pathogenesis, as suggested by human IBD and murine models of intestinal inflammation.<sup>15,16</sup> Dietary therapy can provide clinical benefit in some cases of canine IBD,<sup>17</sup> thus implicating dietary factors in the pathogenesis. Soft Coated

Wheaten Terriers with protein-losing enteropathy develop immune responses to dietary antigens.<sup>25-28</sup>

A number of studies have implicated genetic factors in the pathogenesis of human IBD,<sup>31</sup> but the exact link remains unclear. The strongest genetic associations are those with genes of the human major histocompatibility complex (MHC) (eg,



**Fig 3.** Duodenal mucosa from a dog with eosinophilic enteritis. There is evidence of architectural abnormalities in conjunction with alterations in mucosal immune cell populations, which are predominantly eosinophils. Sirius red.

human leukocyte antigen), whereas some but not all researchers have suggested that intestinal permeability is increased in both patients with Crohn's disease and their healthy relatives.<sup>31,33</sup> There is also recent evidence that a proportion ( $\leq 15\%$ ) of human patients with Crohn's disease have a mutation in the *NOD2* gene on chromosome 16.<sup>34,35</sup> Given that *NOD2* functions to detect bacterial lipopolysaccharide and that it can activate the potent proinflammatory transcription factor NF- $\kappa$ B, a role in disease pathogenesis is possible and would confirm a link between aberrations in immune responses to bacteria and disease expression. The importance of genetic factors in canine IBD is implied by the fact that certain breeds appear predisposed and the fact that certain forms of IBD are

seen only in single breeds or pedigree lines.<sup>17,36</sup> However, detailed studies of genetic linkages or microsatellite markers are lacking in dogs with IBD.

#### *Lymphocytic-Plasmacytic Enteritis and Nonspecific IBD*

Although it has been suggested that the pathogenesis of canine IBD is similar to that of the human diseases, there has been limited work on immunologic mechanisms in canine lymphocytic-plasmacytic enteropathies. Most studies have involved the use of immunohistochemical markers to delineate immune cell populations in tissues collected from

clinical cases. In 1996, Jergens et al<sup>37</sup> documented a decrease in intestinal T cell and IgG<sup>+</sup> plasma cell numbers in dogs with small intestinal IBD. The same authors followed up this study with assessment of large bowel IBD and noted increases in IgA<sup>+</sup> and IgG<sup>+</sup> plasma cells and T cells.<sup>38</sup> Similar findings were documented by Stonehewer et al,<sup>39</sup> who described increased percentages of total lamina propria plasma cells, T cells, and intraepithelial lymphocytes in dogs with colonic IBD. A more recent immunohistochemical study of small intestinal lymphocytic plasmacytic enteritis<sup>40</sup> also documented alterations in mucosal immune cell populations. Increased lamina propria T cell numbers were documented, particularly T cells expressing the  $\alpha\beta$  T-cell receptor and CD4, as were increased IgG<sup>+</sup> plasma cells, macrophages, and granulocytes. The former finding suggests an important role for the CD4<sup>+</sup> T helper cell in IBD pathogenesis, whereas the latter finding suggests the presence of active and ongoing inflammation. Conversely, a decrease in total mast cell numbers was documented, which contrasts with results of another recently published immunohistochemical study, documenting increased numbers of mucosal mast cells and IgE<sup>+</sup> cells in dogs with IBD.<sup>41</sup> This discrepancy would best be explained by the fact that different methods (eg, toluidine blue [TB] vs tryptase and IgE) were used to document mast cells. The latter study likely documented both intact and degranulated populations, whereas the use of TB in the former study meant that only intact mast cells would have been documented. Thus, there is likely an overall increase in this population with increased degranulation, as has been documented previously in human IBD.<sup>42</sup> In the epithelial compartment, increased intraepithelial CD3<sup>+</sup> T lymphocytes have been documented by immunohistochemical techniques.<sup>40</sup> Further, flow cytometric analysis revealed changes in the proportions of intraepithelial lymphocyte subsets, with a decreased percentage of  $\gamma\delta$  T lymphocytes.<sup>43</sup> Although there is some disagreement among the above studies, the overall trends are broadly similar. The differences are most likely the result of differences in methodology, study populations, and the definition of IBD. Future comparisons would be easier if a standard IBD classification scheme were adopted, as previously recommended.<sup>36</sup>

Acute phase proteins may play a role in canine IBD.<sup>44</sup> Increased concentrations of C-reactive protein were documented, which decreased after treatment of IBD. Correlation was also noted between both C-reactive protein and haptoglobin, and canine IBD disease activity indices, suggesting that these proteins may also play a role in diagnosis and monitoring. Nitric oxide is increased in the colonic lavage fluid of dogs with IBD,<sup>45</sup> and increased expression of mRNA encoding the enzyme responsible for nitric oxide production (inducible nitric oxide synthase) has also been documented.<sup>46</sup> However, the significance of such a finding is not clear because there is some debate as to whether nitric oxide plays an anti- or proinflammatory role.<sup>32</sup>

Altered cytokine patterns have recently been documented in both small and large intestine IBD.<sup>47,48</sup> In the diseased small intestine, increased mRNA expression for Th1 (IL-2, IL-12, and interferon [INF]  $\gamma$ ), Th2 (IL-5), proinflammatory (tumor necrosis factor [TNF]  $\alpha$ ), and immunoregulatory (TGF $\beta$ ) cytokines occurs, which is not clearly either

Th1 or Th2 in profile.<sup>47</sup> Large intestinal IBD is broadly similar, with increases in IL-2, IL-12, TNF $\alpha$ , and TGF $\beta$ .<sup>48</sup> Such studies allow a better understanding of canine IBD and will enable us to assess more closely the role of interventional therapies for canine lymphocytic-plasmacytic enteritis. The omega (n-) fatty acids provide substrates for the cyclooxygenase and lipoxygenase pathways and are of importance in the pathogenesis of intestinal inflammation in humans<sup>49</sup> but have not yet been investigated in the canine mucosa.

### *Histiocytic Ulcerative Colitis in Boxer Dogs*

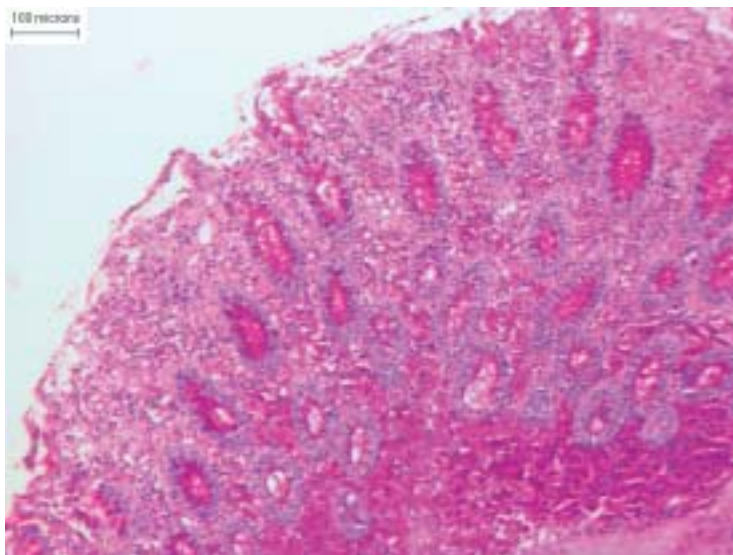
Histiocytic ulcerative colitis (HUC), a particularly severe form of IBD that principally but not exclusively affects young Boxer dogs, was first described over 30 years ago.<sup>50,51</sup> The gross, histopathologic (Fig 4), and ultrastructural changes seen in HUC have been well characterized<sup>50,52-57</sup> and are distinct from those changes associated with other idiopathic canine colitides. The presence within the lesions of large macrophages that are strongly periodic acid-Schiff positive is pathognomonic for HUC.<sup>50,52,53</sup>

The etiology of HUC remains poorly characterized. Some authors have suggested a role for an infectious agent, given the presence of the periodic acid-Schiff-positive macrophages. These macrophages are also characteristic of Whipple's disease in humans and Johne's disease in cattle, which both have an infectious cause.<sup>58</sup> Although a possible role for infectious organisms has been suggested,<sup>50,55,59</sup> a single consistent etiologic agent has not been implicated.<sup>54,60</sup> Attempts at experimentally reproducing the pathologic changes through infection with mycoplasmas has been unsuccessful.<sup>61</sup> Therefore, intralumenal bacteria are likely to be secondary invaders that exacerbate existing mucosal inflammation, as documented both for human IBD and murine models of chronic intestinal inflammation.<sup>31</sup>

An alternative hypothesis is that the periodic acid-Schiff-positive macrophages are directly connected to the disease etiopathogenesis. However, these cells appear late in the disease process, and the early lesions are characterized by epithelial defects with secondary cellular infiltration.<sup>54,62</sup> Thus, epithelial damage is likely to be the initial inciting event, but the exact pathogenetic mechanism remains to be elucidated. Nevertheless, immune cell populations are likely to be involved in the pathogenesis of HUC.<sup>63</sup> Marked increases in IgG<sup>+</sup> plasma cells, especially of the IgG3 and IgG4 subclasses, occurs, broadly similar to the the situation in human ulcerative colitis.<sup>64-66</sup> T cells, MHC class II<sup>+</sup> cells, macrophages, and granulocytes are all increased within the lamina propria. There is also increased expression of MHC class II molecules by the intestinal epithelium, which is again similar to findings in human IBD.<sup>67</sup> The function of MHC class II molecule expression by enterocytes is not known, although these cells can present soluble antigen to T cells *in vitro*.<sup>68</sup> Given that such a process may induce immunologic tolerance,<sup>68</sup> the documented increase in epithelial MHC class II expression may be an attempt to down-regulate aberrant mucosal immune responses.

### *Immunoproliferative Enteropathy of Basenji Dogs*

This condition was first recognized by Fox et al in 1965<sup>69</sup> and is thought to be similar to immunoproliferative enter-



**Fig 4.** Colonic mucosa from a Boxer with histiocytic ulcerative colitis. There is marked epithelial loss and evidence of a mixed immune cell infiltrate, which includes large numbers of periodic acid-Schiff–positive histiocytes.

opathy in humans.<sup>70</sup> However, the canine disease is more diverse and involves other organ systems in addition to the gastrointestinal tract. A strong genetic component to the pathogenesis of this disease has been recognized, and pedigree analysis has suggested an autosomal recessive mode of inheritance.<sup>22</sup>

Pathologic changes involve the stomach, where there is gastric mucosal hypertrophy, lymphoid cell infiltration, hyperplasia of fundic gland parietal and chief cells, and ulceration.<sup>19–21</sup> The whole of the small intestine can be involved, but the most severe lesions are proximal. Histopathologic findings include villous blunting, crypt elongation, and infiltration of the lamina propria by lymphocytes, plasma cells, and occasionally neutrophils. Mast cell numbers are reduced, and electron microscopy studies have revealed evidence of degranulation.<sup>71</sup> There are increases in lamina propria plasma cells of all classes.<sup>21</sup>

The most striking immune system alteration is the marked polyclonal increase in serum IgA.<sup>70</sup> However, there is no evidence for increased  $\alpha$ -chain or heavy chain fragments, as seen in humans with immunoproliferative small intestinal disease. Further, IgA concentrations in intestinal washes were not significantly increased.<sup>70</sup> There was no correlation between changes in lymphocyte blastogenesis and progression of disease severity.<sup>72</sup> The requirement for immunosuppressive doses of prednisolone to control clinical signs confirms a role for the immune system in pathogenesis, whereas dietary factors are thought to be less important based on the fact that dietary manipulation has little effect.<sup>73</sup>

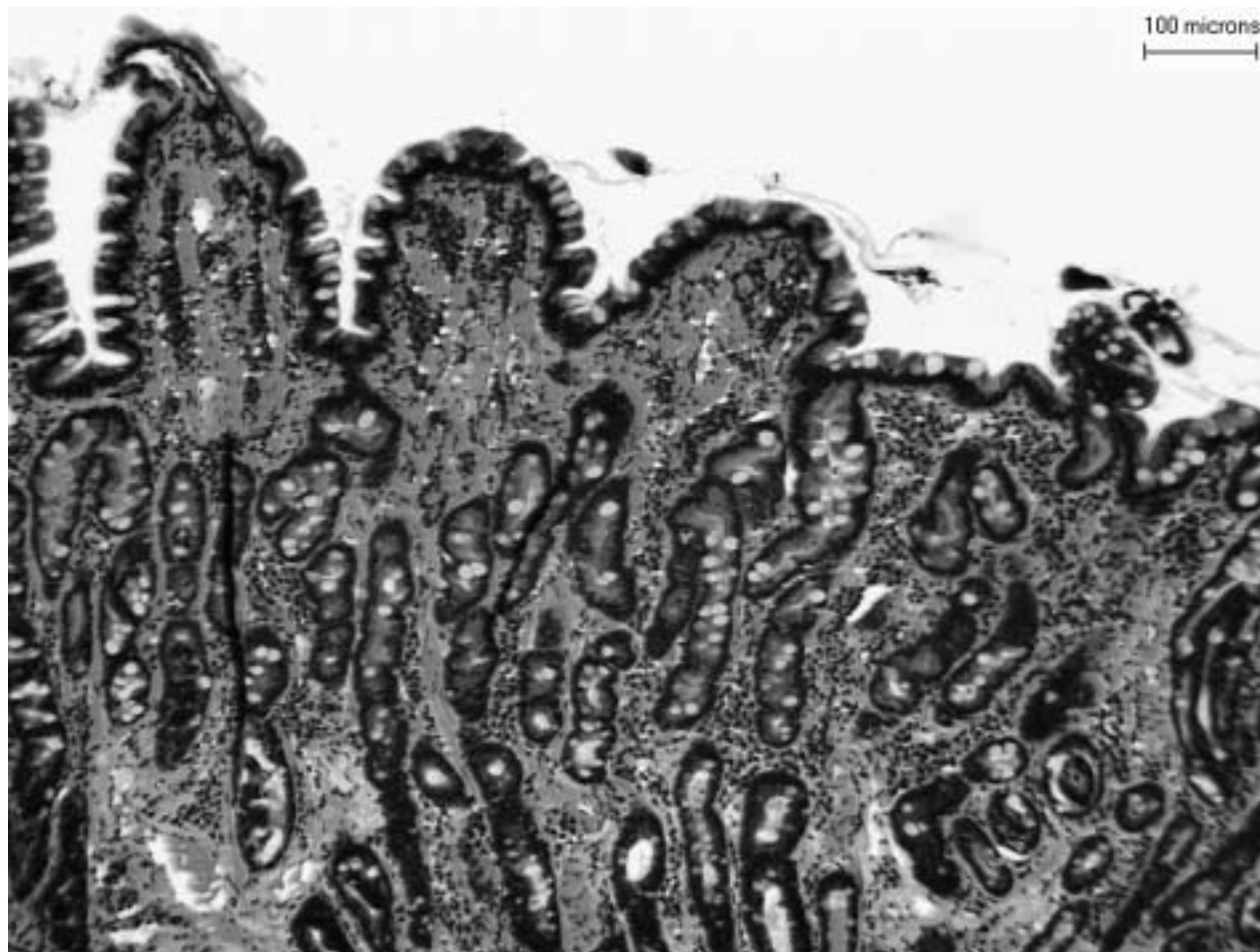
#### ***Familial Protein-Losing Enteropathy and Protein-Losing Nephropathy in Soft Coated Wheaten Terriers***

Recently, a clinical syndrome unique to Soft Coated Wheaten Terriers has been characterized.<sup>25–28</sup> Affected dogs present with signs of protein-losing enteropathy, protein-

losing nephropathy, or both. The pattern of disease expression implies a genetic basis, but the mode of inheritance is not yet known. However, pedigree analysis of 188 dogs demonstrated a common male ancestor.<sup>28</sup>

Histopathologic changes are consistent with IBD (inflammatory cell infiltrates, villous blunting, and epithelial erosions) and with lymphatic dilation, the latter seen in lamina propria and submucosa.<sup>26</sup> Inflammatory cell infiltrates usually consist of lymphocytes and plasma cells, but neutrophils and eosinophils are often present and may predominate.

This condition has yet to be completely assessed at the immunologic level. However, the potential role for food hypersensitivity as a component of pathogenesis has recently been examined.<sup>26,28</sup> In 1 study, 6 Soft Coated Wheaten Terriers with protein-losing enteropathy or protein-losing nephropathy were evaluated by gastroscopic food-sensitivity testing, provocative dietary trials, and an assay of fecal IgE response to specific food allergens.<sup>26</sup> Positive responses were elicited by gastroscopic food-sensitivity testing to a variety of food antigens in 5 of 6 dogs. Further, all 6 dogs demonstrated adverse reactions during provocative food trials, as determined by diarrhea, vomiting, or pruritus. Moreover, there were concurrent reductions in serum albumin and increases in fecal  $\alpha_1$ -protease inhibitor during these adverse reactions. There were also variations in antigen-specific fecal IgE concentrations throughout the trial. A further study assessed intestinal permeability, gluten sensitivity, and the numbers of intestinal eosinophils, lymphocytes, and plasma cells in affected Soft Coated Wheaten Terriers.<sup>28</sup> Intestinal permeability did not differ significantly between Soft Coated Wheaten Terriers and control dogs, although the administration of gluten to affected Soft Coated Wheaten Terriers resulted in a significant decrease in globulin concentrations, suggesting some protein loss. In affected Soft Coated Wheaten Terriers, eosinophils, lymphocytes, and plasma cells were increased above normal, based on



**Fig 5.** Duodenal mucosa from an Irish Setter with gluten-sensitive enteropathy. The predominant finding is marked villus atrophy. Hematoxylin and eosin.

previously published control values.<sup>74</sup> After gluten administration, the authors reported trends for increased mucosal infiltration of lymphocytes and plasma cells, and reduced numbers of eosinophils were noted. However, none of these results achieved significance and would have been within normal limits if based on other published quantitative values for immune cell numbers in control dogs.<sup>75</sup> This discrepancy may be the result of the study populations examined and techniques used for cell counting. Inclusion of a contemporary control group, to enable direct comparisons of mucosal immune cell counts with affected dogs, will allow these findings to be clarified. These interesting preliminary results suggest an underlying immune-based etiology, which should be assessed in more detail in future studies.

### Gluten-Sensitive Enteropathy in Irish Setters

Gluten-sensitive enteropathy is a disease of the small intestine that has been documented in Irish Setters and is caused by exposure of affected individuals to a diet containing wheat gluten.<sup>76-79</sup> This familial condition most likely has an autosomal recessive mode of inheritance,<sup>80</sup> but unlike human celiac disease, there is no relationship with major histocompatibility genes *DQA* and *DQB*.<sup>81</sup> Pathology

may be age dependent in some dogs, who may no longer respond to gluten as adults after demonstrating gluten sensitivity when younger.<sup>82</sup> The reason for such age dependence has not yet been evaluated in detail.

Histopathologic examination of the small intestine reveals villous atrophy (Fig 5), increases in intraepithelial lymphocyte numbers, and a variable inflammatory cell infiltration of the lamina propria. Abnormal mucosal permeability exists in affected dogs, and precedes the development of disease.<sup>83</sup> Immunohistochemical studies have demonstrated increased lamina propria CD4<sup>+</sup> and decreased CD8<sup>+</sup> T-cell populations in affected dogs.<sup>84</sup> During recovery from disease, there are increases in CD3<sup>+</sup> and CD8<sup>+</sup> lamina propria lymphocytes and intraepithelial lymphocytes. Mucosal and systemic immune responses to gluten have also been assessed in affected dogs.<sup>85,86</sup> Challenge of gluten-sensitive enteropathy dogs with gluten causes increases in circulating CD4<sup>+</sup> lymphocytes and granulocytes. These changes are different from the responses of control Irish Setters, suggesting a proinflammatory rather than tolerogenic response.

Irish Setters with gluten-sensitive enteropathy also have increased serum total IgA concentrations but no differences in responses of any immunoglobulin class to ovalbumin,

collagens I and II, and soya compared with normal dogs.<sup>87</sup> Decreased serum anti-gliadin immunoglobulin concentrations were noted,<sup>87</sup> but there were no alterations in serum immunoglobulin responses to gluten or endomysial antigens, as seen for human celiac disease. Therefore, the pathogenesis of Irish Setter gluten-sensitive enteropathy remains incompletely characterized. The lack of linkage to MHC haplotypes and differences in the humoral immune response suggest mechanisms distinct from those underlying human celiac disease.

### Idiopathic Small Intestinal Bacterial Overgrowth

Small intestinal bacterial overgrowth is defined as an increase in the number of bacteria in the small intestine above that considered normal<sup>88</sup> and has been proposed as a cause of clinical signs of diarrhea and weight loss. Small intestinal bacterial overgrowth may arise secondary to an underlying disorder, such as a partial intestinal obstruction, or may occur as a primary (idiopathic) condition. The idiopathic form of the disease is controversial because its etiology and pathogenesis and the role of enteric flora remain poorly defined. Hall and Simpson<sup>88</sup> recommended that idiopathic small intestinal bacterial overgrowth be renamed antibiotic-responsive diarrhea until more is known about its pathogenesis.

The condition predominantly affects young animals, and the German Shepherd Dog is predisposed.<sup>89</sup> The reason for such a predisposition is not clear but may be related to the concurrence of IgA deficiency in this breed. In this regard, some studies have suggested a relative serum IgA deficiency in healthy German Shepherd Dogs,<sup>90,91</sup> whereas in other studies serum IgA concentrations were more consistent with IgA dysregulation.<sup>92</sup> Differences in the findings among studies are likely due to differences in study populations and methodology, as previously discussed.<sup>93</sup> Further, recent reports have demonstrated low tear and fecal IgA concentrations in healthy German Shepherd Dogs, suggesting defective mucosal IgA secretion in this breed.<sup>94,95</sup> German Shepherd Dogs with documented small intestinal bacterial overgrowth (antibiotic-responsive diarrhea) may have relatively low serum and duodenal juice IgA concentrations,<sup>91,96,97</sup> and there is a relative deficiency of IgA secretion from the small intestinal mucosa of German Shepherd Dogs with small intestinal diseases.<sup>93</sup> However, duodenal IgA<sup>+</sup> plasma cell numbers in German Shepherd Dogs with antibiotic-responsive diarrhea are either normal<sup>91</sup> or increased.<sup>40</sup> Again, methodologic differences likely account for the discrepancy among these studies. A recent study of a German Shepherd Dog colony revealed an association of reduced serum and fecal IgA concentrations, increased serum IgG concentrations, and colonization of the gut by enteropathogenic *Escherichia coli*.<sup>98</sup> These findings are consistent with a defective mucosal barrier leading to increased systemic responsiveness.

A recent immunohistochemical study of immune cell populations in the duodenal mucosa of dogs with antibiotic-responsive diarrhea has demonstrated that although minimal histopathologic changes exist, there are increases in numbers of lamina propria CD4<sup>+</sup> cells in affected dogs.<sup>40</sup> This

finding may suggest a role for the mucosal immune system in pathogenesis of disease. In this regard, increased mucosal expression of mRNA encoding a variety of cytokines (but most notably TNF $\alpha$  and TGF $\beta$ ) has also been documented in dogs with antibiotic-responsive diarrhea.<sup>47</sup> In situ hybridization techniques have demonstrated increases in IL-10 and IFN $\gamma$  mRNA expression in dogs with numerically larger duodenal bacterial populations.<sup>99</sup>

Although antibiotic treatment may lead to resolution of clinical signs, the true effect of antibiotics on small intestinal bacteria is not clear. In one study, there was a variable effect of antibiotics,<sup>100</sup> but more recently German Shepherd Dogs with small intestinal bacterial overgrowth that were treated with oxytetracycline demonstrated resolution of clinical signs and reductions in cytokine mRNA for TNF $\alpha$  and TGF $\beta$ , but bacterial numbers (both total and anaerobic) did not decline.<sup>47</sup> Given that a sterilizing effect on the small intestine is unlikely, the antibiotics may instead provide a selective pressure on the intestinal flora, encouraging the establishment of less harmful bacteria at the expense of more pathogenic species. In this regard, recent work in a mouse model of intestinal inflammation suggests that individual bacterial species of the resident flora differ in immunogenicity.<sup>101</sup> Alternatively, these drugs are known to have immunomodulatory effects, which may be an important mode of action.<sup>102-104</sup> Antibiotics also may work directly on the intestinal mucosa to repair the reported biochemical defects<sup>100,105,106</sup> or to restore normal mucosal permeability.<sup>107</sup> Such an effect would reduce the passage of proinflammatory bacterial products into the intestinal mucosa and would explain the reduction in TNF $\alpha$  transcription. Antibiotic therapy may also eliminate an occult pathogen, such as enteropathogenic *E coli*<sup>108-110</sup>; German Shepherd Dogs with mucosal IgA deficiency are predisposed to infection with this pathogen.<sup>95,98</sup>

### Novel Therapies for Canine Enteropathies

In the previous sections, we have addressed immunopathogenetic mechanisms responsible for canine IBD and other enteropathies. Such information coupled with that extrapolated from human IBD and from rodent models of intestinal inflammation should allow the development of more specific treatments. Novel treatments already at the stage of clinical trials in humans include new immunosuppressive drugs, drugs that target the actions and function of TNF $\alpha$ , and monoclonal antibody therapy.

Novel immunosuppressive agents include cyclosporin, mycophenolate mofetil, and thalidomide. Cyclosporin is a cyclic peptide produced by the soil fungus *Tolypocladium flatum gams* that specifically affects T lymphocyte functions by a variety of mechanisms, including interference with IL-2 transcription and induction of T lymphocytes that suppress cytotoxic responses.<sup>111-116</sup> Studies on human IBD suggest variable efficacy.<sup>117-120</sup> Because increased T lymphocyte numbers are documented in canine IBD,<sup>40</sup> this drug may be of benefit, but clinical trials will be required to assess efficacy.

Mycophenolate mofetil has also recently been used in human IBD.<sup>121-126</sup> It is converted to mycophenolic acid, which inhibits type II inosine monophosphate dehydroge-



nase, thereby suppressing guanine synthesis.<sup>126</sup> Because T and B lymphocytes cannot utilize salvage pathways for purine synthesis, they are particularly susceptible. Trials in human medicine have again had a variable outcome,<sup>122,125,126</sup> and this agent does not appear to be more effective than existing drugs such as azathioprine.<sup>125</sup> Given that lymphocytes are specifically targeted, an indication for canine IBD would be suggested, although this agent has yet to undergo clinical trials.

A number of novel therapies are known to target TNF $\alpha$ , including thalidomide, oxpentifylline, and anti-TNF $\alpha$  monoclonal antibodies. Thalidomide has a multitude of effects in addition to inhibition of TNF $\alpha$  expression, including the reduction of IL-12 expression, reduction of leukocyte migration, and impaired angiogenesis.<sup>127-130</sup> Recent open label trials have demonstrated beneficial effects in refractory Crohn's disease,<sup>131,132</sup> although double-blind placebo controlled trials are needed before this agent can be recommended. Thalidomide may be suitable for use in canine IBD mostly because of the importance of cytokines such as IL-12 and TNF $\alpha$  in disease pathogenesis. Oxpentifylline (pentoxifylline) is a phosphodiesterase inhibitor that generates high intracellular cyclic AMP, which then inhibits TNF $\alpha$  expression.<sup>133,134</sup> In vitro studies suggested the potential for beneficial effects in Crohn's disease,<sup>135</sup> but clinical results were less rewarding.<sup>136</sup> An anti-TNF $\alpha$  monoclonal antibody has also been used successfully to treat human IBD.<sup>137,138</sup> A double-blind placebo-controlled multicenter trial demonstrated significant clinical benefits, but endoscopic examination suggested resolution of inflammatory cell infiltrate without improvement in architectural abnormalities in Crohn's disease patients.<sup>137</sup> A similar approach may prove suitable for canine IBD, once a species-specific monoclonal antibody becomes available. An anti-CD4 monoclonal antibody has been useful in the treatment of human IBD.<sup>139</sup> Because CD4<sup>+</sup> T cells are involved in the pathogenesis of canine IBD, such an approach may be beneficial. However, because different CD4<sup>+</sup> T-cell subsets can have either immunogenic or tolerogenic properties, the overall effect would depend upon which subsets were most sensitive.

Dietary therapy is of benefit in a number of chronic canine enteropathies, and recent innovations include the use of hydrolyzed protein diets and manipulation of the n-3 : n-6 fatty acid ratio. Hydrolyzed protein diets, usually based on either chicken or soy protein, have already been developed and marketed for dogs and would be of benefit when pathogenesis includes adverse reactions to dietary components. Because these diets include protein derivatives of lower molecular mass than that proposed for food antigens (10–70 kD),<sup>140</sup> they would be expected to be hypoallergenic. However, there is little objective information to confirm this hypothesis, in part because it is unclear as to what target molecular mass would guarantee that a protein is rendered hypoallergenic.<sup>141</sup> There is also concern that the chemical digestion process may expose hidden antigenic epitopes,<sup>142</sup> allowing novel adverse immunologic responses to develop. In a recent clinical trial, a hydrolyzed diet did elicit some beneficial response in dogs with refractory IBD,<sup>142</sup> but more studies are required to confirm this effect. Manipulation of the n-3 : n-6 ratio has been beneficial in

some human inflammatory diseases, including atopic dermatitis and rheumatoid arthritis.<sup>143,144</sup> However, results in human IBD are more variable, with some studies demonstrating favorable responses<sup>145,146</sup> while others are less favorable.<sup>147</sup> Diets with modified n-3 : n-6 ratios are already being used for dogs with gastrointestinal diseases, but there is little objective information on their efficacy.

Probiotics and prebiotics have the potential to modify the intestinal microflora, with proposed benefits for patients with enteropathies. A probiotic is defined as a living organism that upon ingestion in certain numbers exerts health benefits beyond those of inherent basic nutrition.<sup>148</sup> In addition to direct antagonistic effects on pathogenic bacteria, probiotics modulate mucosal immune responses, either by stimulating innate (eg, phagocytic activity) or specific (eg, secretory IgA) immune responses.<sup>149</sup> *Lactobacillus* species are commonly used as probiotics, but because different *Lactobacillus* species generate either Th1 or Th2 cytokine patterns,<sup>150</sup> care should be taken to select the most appropriate organisms. Prebiotics are selective substrates for a limited number of "beneficial" species and therefore cause alterations in the luminal microflora.<sup>150</sup> The most frequently used prebiotics are nondigestible carbohydrates, such as lactulose, inulin, and fructo-oligosaccharides.<sup>96,151,152</sup> Fructo-oligosaccharides have reduced intestinal colonization by *Salmonella* species in poultry<sup>153</sup> and altered intestinal bacteria in humans.<sup>154</sup> Results of a recent study suggested that fructo-oligosaccharides might alter the nature of the small intestinal microflora of healthy German Shepherd Dogs with small intestinal bacterial overgrowth.<sup>96</sup> However, the significance of these changes is unclear because greater alterations were found in the control group. More recent work in cats has shown that addition of fructo-oligosaccharides to diets alters the colonic but not the small intestinal microbiota.<sup>155</sup>

Both probiotics and prebiotics can reduce intestinal inflammation in mouse models of IBD<sup>156,157</sup> and be important future therapeutic modalities for human IBD patients. Probiotic bacterial strains are currently part of placebo-controlled trials for treatment of human IBD.<sup>147</sup> Probiotic preparations and diets containing prebiotics are marketed for use in dogs, and the conditions most likely to benefit from their use would include IBD and antibiotic-responsive diarrhea.

## Summary

The immunopathogenetic mechanisms underlying human IBD and murine models of intestinal inflammation have been well described. Although canine mucosal immunology is still in its infancy, we are beginning to understand better the underlying cause of enteropathies such as IBD and antibiotic-responsive diarrhea. Knowledge of these mechanisms will allow us to design strategies for more appropriate treatment.

## References

1. Elson CO. Experimental models of intestinal inflammation: New insights into mechanisms of mucosal homeostasis. In: Ogra PL, Messtecky J, Lamm ME, et al, eds. *Mucosal Immunology*, 2nd ed. San Diego, CA: Academic Press; 1999:1007–1034.
2. Hermiston ML, Gordon JI. Inflammatory bowel disease and ad-

- enomas in mice expressing a dominant negative N-cadherin. *Science* 1995;270:1203–1207.
3. Kundig TM, Schorle H, Bachmann MF, et al. Immune responses in interleukin-2-deficient mice. *Science* 1993;262:1059–1061.
  4. Kuhn R, Lohler J, Rennick D, et al. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993;75:263–274.
  5. Watanabe M, Ueno Y, Yajima T, et al. Interleukin 7 transgenic mice develop chronic colitis with decreased interleukin 7 protein accumulation in the colonic mucosa. *J Exp Med* 1998;187:389–402.
  6. Morrissey PJ, Charrier K, Braddy S, et al. CD4<sup>+</sup> T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified CD4<sup>+</sup> T cells. *J Exp Med* 1993;178:237–244.
  7. Powrie F, Mason D. Ox-22<sup>high</sup> CD4<sup>+</sup> T cells induce wasting disease with multiple organ pathology: Prevention by the Ox-22<sup>low</sup> subset. *J Exp Med* 1990;172:1701–1708.
  8. Powrie F, Leach MW, Mauze S, et al. Phenotypically distinct subsets of CD4<sup>+</sup> T cells induce or protect from chronic intestinal inflammation in C.B-17 scid mice. *Int Immunol* 1993;5:1461–1471.
  9. Powrie F, Leach MW, Mauze S, et al. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RB<sup>hi</sup> CD4<sup>+</sup> T cells. *Immunity* 1994;1:553–562.
  10. Powrie F, Carlino J, Leach MW, et al. A critical role for transforming growth factor- $\beta$  but not interleukin 4 in the suppression of T helper type-1 mediated colitis by CD45RB<sup>low</sup> CD4<sup>+</sup> T cells. *J Exp Med* 1996;183:2669–2674.
  11. Bonhagen K, Thoma S, Bland PW, et al. Cytotoxic reactivity of gut lamina propria CD4<sup>+</sup>  $\alpha\beta$  T cells in SCID mice with colitis. *Eur J Immunol* 1996;26:3074–3083.
  12. Bonhagen K, Thoma S, Leithauser F, et al. A pancolitis resembling human ulcerative colitis (UC) is induced by CD4<sup>+</sup> TCR  $\alpha\beta$  T cells of athymic origin in histocompatible severe combined immunodeficient (SCID) mice. *Clin Exp Immunol* 1998;112:443–452.
  13. Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25<sup>+</sup>CD4<sup>+</sup> regulatory cells that control intestinal inflammation. *J Exp Med* 2000;192:295–302.
  14. Takahashi T, Tagami T, Yamazaki S, et al. Immunologic self-tolerance maintained by CD25<sup>+</sup>CD4<sup>+</sup> regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med* 2000;192:303–309.
  15. Duchmann R, Kaiser I, Hermann E, et al. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). *Clin Exp Immunol* 1995;102:448–455.
  16. Duchmann R, Schmitt E, Knolle P, et al. Tolerance towards resident intestinal flora in mice is abrogated in experimental colitis and restored by treatment with interleukin-10 or antibodies to interleukin-12. *Eur J Immunol* 1996;26:934–938.
  17. Guilford WG. Idiopathic inflammatory bowel diseases. In: Guilford WG, Center SA, Strombeck DR, et al, eds. *Strombeck's Small Animal Gastroenterology*, 3rd ed. Philadelphia, PA: WB Saunders; 1996:451–486.
  18. Hall EJ, Rutgers HC, Scholes SFE, et al. Histiocytic ulcerative colitis in Boxer dogs in the UK. *J Small Anim Pract* 1994;35:509–515.
  19. Breitschwerdt EB, Waltman C, Hagstad HV, et al. Clinical and epidemiologic characterization of a diarrheal syndrome in Basenji dogs. *J Am Vet Med Assoc* 1982;180:914–920.
  20. Breitschwerdt EB, Ochoa R, Barta M, et al. Clinical and laboratory characterization of Basenji dogs with immunoproliferative small intestinal disease. *Am J Vet Res* 1984;45:267–273.
  21. MacLachlan NJ, Breitschwerdt EB, Chambers JM, et al. Gastroenteritis of Basenji dogs. *Vet Pathol* 1988;25:36–41.
  22. Breitschwerdt EB, Halliwell WH, Foley CW, et al. An hereditary diarrhetic syndrome in the Basenji characterized by malabsorption, protein-losing enteropathy and hypergammaglobulinemia. *J Am Anim Hosp Assoc* 1980;16:551–560.
  23. Breitschwerdt EB. Immunoproliferative enteropathy of Basenjis. *Semin Vet Med Surg Small Anim* 1992;7:153–161.
  24. Flesja K, Yri T. Protein-losing enteropathy in the Lundehund. *J Small Anim Pract* 1977;18:11–23.
  25. Littman MP, Giger U. Familial protein-losing enteropathy (PLE) and/or protein-losing nephropathy (PLN) in Soft-Coated Wheaten Terriers (SCWT). *J Vet Intern Med* 1990;4:133.
  26. Vaden SL, Hammerberg B, Davenport DJ, et al. Food hypersensitivity reactions in Soft Coated Wheaten Terriers with protein-losing enteropathy or protein-losing nephropathy or both: Gastroscopic food sensitivity testing, dietary provocation, and fecal immunoglobulin E. *J Vet Intern Med* 2000;14:60–67.
  27. Littman MP, Dambach DM, Vaden SL, Giger U. Familial protein-losing enteropathy and protein-losing nephropathy in Soft Coated Wheaten Terriers: 222 cases (1983–1997). *J Vet Intern Med* 2000;14:68–80.
  28. Vaden SL, Sellon RK, Melgarejo LT, et al. Evaluation of intestinal permeability and gluten sensitivity in Soft-Coated Wheaten Terriers with familial protein-losing enteropathy, protein-losing nephropathy, or both. *Am J Vet Res* 2000;61:518–524.
  29. Barkin R, Lewis JH. Overview on inflammatory bowel disease in humans. *Semin Vet Med Surg* 1992;7:117–127.
  30. Morson BC, Dawson IMP. The small intestine. In: Symms WSC, ed. *Systemic Pathology, Vol 3: Alimentary System*. Edinburgh: Churchill Livingstone; 1978:1054–1098.
  31. Fiocchi C. Inflammatory bowel disease: Etiology and pathogenesis. *Gastroenterology* 1998;115:182–205.
  32. Karp LC, Targan SR. Ulcerative colitis: Evidence for an updated hypothesis of disease pathogenesis. In: Ogra PL, Mestecky J, Lamm ME, et al, eds. *Mucosal Immunology*, 2nd ed. San Diego, CA: Academic Press; 1999:1047–1053.
  33. Duchmann R, Zeitz M. Crohn's disease. In: Ogra PL, Mestecky J, Lamm ME, et al, eds. *Mucosal Immunology*, 2nd ed. San Diego, CA: Academic Press; 1999:1055–1080.
  34. Hugot JP, Chamaillard M, Zouali H, et al. Association of *NOD2* leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001;411:599–603.
  35. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in *NOD2* associated with susceptibility to Crohn's disease. *Nature* 2001;411:603–606.
  36. Jergens AE. Inflammatory bowel disease—Current perspectives. *Vet Clin North Am* 1999;29:501–521.
  37. Jergens AE, Moore FM, Kaiser MS, et al. Morphometric evaluation of immunoglobulin A-containing and immunoglobulin G-containing cells and T cells in duodenal mucosa from healthy dogs and from dogs with inflammatory bowel disease or non-specific gastroenteritis. *Am J Vet Res* 1996;57:697–704.
  38. Jergens AE, Gamet Y, Moore FM, et al. Colonic lymphocyte and plasma cell populations in dogs with lymphocytic-plasmacytic colitis. *Am J Vet Res* 1999;60:515–520.
  39. Stonehewer J, Simpson JW, Else RW, Macintyre N. Evaluation of B and T lymphocytes and plasma cells in colonic mucosa from healthy dogs and from dogs with inflammatory bowel disease. *Res Vet Sci* 1998;65:59–63.
  40. German AJ, Hall EJ, Day MJ. Immune cell populations within the duodenal mucosa of dogs with enteropathies. *J Vet Intern Med* 2001;15:14–25.
  41. Locher C, Tipold A, Welle M, et al. Quantitative assessment of mast cells and expression of IgE protein and mRNA for IgE and interleukin 4 in the gastrointestinal tract of healthy dogs and dogs with inflammatory bowel disease. *Am J Vet Res* 2001;62:211–216.
  42. Knutson L, Ahrenstedt O, Odland B, Hallgren R. The jejunal secretion of histamine is increased in active Crohn's disease. *Gastroenterology* 1990;98:849–854.
  43. Jergens AE, Sonea IM, Kaufman LK, et al. Flow cytometric

- analysis of mucosal lymphocytes in dogs with inflammatory bowel disease. *J Vet Intern Med* 2001;15:275.
44. Jergens AE, Schreiner CA, Ahrens FA, et al. Laboratory assessment of disease activity in canine inflammatory bowel disease. *J Vet Intern Med* 2000;14:347.
  45. Gunawardana SC, Jergens AE, Ahrens FA, Niyo Y. Colonic nitrite and immunoglobulin G concentrations in dogs with inflammatory bowel disease. *J Am Vet Med Assoc* 1997;211:318–321.
  46. Jergens AE, Carpenter SL, Wannemuehler Y, Ahrens FA. Molecular detection of inducible nitric oxide synthase in canine inflammatory bowel disease. *J Vet Intern Med* 1998;12:205.
  47. German AJ, Helps CR, Hall EJ, Day MJ. Cytokine mRNA expression in mucosal biopsies from German Shepherd Dogs with small intestinal enteropathies. *Digest Dis Sci* 2000;45:7–17.
  48. Ridyard AE, Nuttall TJ, Else RW, et al. Cytokine mRNA expression in the colonic mucosa of dogs with idiopathic colitis. Proceedings of the European Society for Veterinary Internal Medicine Congress, Dublin, Ireland, 2001:54–55.
  49. Schleuren M, Dais W, Steinhilber D, et al. Effects of long-term application of fish oil on patients with Crohn's disease. *Scand J Gastroenterol* 1989;24(Suppl 158):100–101.
  50. van Kruiningen HJ, Montali RJ, Strandberg JD, Kirk RW. A granulomatous colitis of dogs with histological resemblance to Whipple's disease. *Pathol Vet* 1965;2:521–544.
  51. Stokes JE, Kruger JM, Mullaney T, et al. Histiocytic ulcerative colitis in three non-Boxer dogs. *J Am Anim Hosp Assoc* 2001;37:461–465.
  52. Kennedy PC, Cello RM. Colitis of Boxer dogs. *Gastroenterology* 1966;51:926–931.
  53. Sander CH, Langham RF. Canine histiocytic ulcerative colitis. A condition resembling Whipple's disease, colonic histiocytosis, and malakoplakia in man. *Arch Pathol* 1968;85:94–100.
  54. Russell SW, Gomez JA, Trowbridge JO. Canine histiocytic ulcerative colitis. The early lesion and its progression to ulceration. *Lab Invest* 1971;25:509–515.
  55. Lindberg R, Segall T. Histiocytic ulcerative colitis in a Boxer. A case report. *Nord Veterinaarmed* 1977;29:552–555.
  56. Hill FWG, Sullivan ND. Histiocytic ulcerative colitis in a Boxer dog. *Aust Vet J* 1978;54:447–449.
  57. Churcher RK, Watson ADJ. Canine histiocytic ulcerative colitis. *Aust Vet J* 1997;75:710–713.
  58. van Kruiningen HJ. Granulomatous colitis of Boxer dogs: Comparative aspects. *Gastroenterology* 1967;53:114–122.
  59. van Kruiningen HJ. The ultrastructure of macrophages in granulomatous colitis of Boxer dogs. *Vet Pathol* 1975;12:446–459.
  60. Cockrell BY, Krehbiel JD. Ultrastructural changes in histiocytic ulcerative colitis in a Boxer. *Am J Vet Res* 1972;33:453–459.
  61. Bowe PS, van Kruiningen HJ, Rosendal S. Attempts to produce granulomatous colitis in Boxer dogs with a mycoplasma. *Can J Comp Med* 1982;46:430–433.
  62. Gomez JA, Russell SW, Trowbridge JO, Lee J. Canine histiocytic ulcerative colitis—An ultrastructural study of the early mucosal lesion. *Digest Dis* 1977;22:485–496.
  63. German AJ, Hall EJ, Kelly DF, et al. An immunohistochemical study of histiocytic ulcerative colitis in Boxer dogs. *J Comp Pathol* 2000;122:163–175.
  64. Helgeland L, Tysk C, Jarnerot G, et al. IgG subclass distribution in serum and rectal mucosa of monozygotic twins with or without inflammatory bowel disease. *Gut* 1992;33:1358–1364.
  65. Ruthlein J, Ibe M, Burghardt W, et al. Immunoglobulin-G (IgG), IgG1, and IgG2 determinations from endoscopic biopsy specimens in control, Crohn's disease and ulcerative colitis subjects. *Gut* 1992;33:507–512.
  66. Arato A, Savilahti E. IgG3 and IgG4 cells are increased in active ulcerative colitis. *Digestion* 1990;47:35–41.
  67. Mayer L, Eisenhardt D, Solomon P, et al. Expression of class II molecules on intestinal epithelial cells in humans—Differences between normal and inflammatory bowel disease. *Gastroenterology* 1991;100:3–12.
  68. Bland PW, Warren LG. Antigen presentation by epithelial cells of the rat small intestine. I. Kinetics, antigen specificity and blocking by anti-Ia antisera. *Immunology* 1986;58:1–7.
  69. Fox IW, Hoag WG, Strout J. Breed susceptibility, pathogenicity and epidemiology of endemic coliform enteritis in the dog. *Lab Anim Care* 1965;15:194–200.
  70. De Buysscher EV, Breitschwerdt EB, MacLachlan NJ. Elevated serum IgA associated with immunoproliferative enteropathy of Basenji dogs: Lack of evidence for alpha-heavy-chain disease or enhanced intestinal IgA secretion. *Vet Immunol Immunopathol* 1988;20:41–52.
  71. Ochoa R, Breitschwerdt EB, Lincoln KL. Immunoproliferative small intestinal disease in Basenji dogs: Morphologic implications. *Am J Vet Res* 1984;45:482–490.
  72. Barta O, Breitschwerdt EB, Shaffer LM, Pourciau SS. Lymphocyte transformation and humoral immune factors in Basenji dogs with immunoproliferative small intestinal disease. *Am J Vet Res* 1983;44:1954–1959.
  73. Breitschwerdt EB, Hiraakawa DA, Hurlbert SA, et al. Effects of dietary protein source on Basenjis with immunoproliferative enteropathy. *Am J Vet Res* 1992;53:234–236.
  74. Yamasaki K, Suematsu H, Takahashi T. Comparison of gastric and duodenal lesions in dogs and cats with and without lymphocytic-plasmacytic enteritis. *J Am Vet Med Assoc* 1996;209:95–97.
  75. German AJ, Hall EJ, Day MJ. Analysis of leucocyte subsets in the canine intestine. *J Comp Pathol* 1999;120:129–145.
  76. Batt RM, Carter MW, McLean L. Morphological and biochemical studies of a naturally occurring enteropathy in the Irish Setter dog: A comparison with coeliac disease in man. *Res Vet Sci* 1984;37:339–346.
  77. Batt RM, Carter MW, McLean L. Wheat-sensitive enteropathy in Irish Setter dogs: Possible age-related brush border abnormalities. *Res Vet Sci* 1985;39:80–83.
  78. Batt RM, McLean L, Carter MW. Sequential morphological and biochemical studies of naturally occurring wheat-sensitive enteropathy in Irish Setter dogs. *Digest Dis Sci* 1987;32:184–194.
  79. Hall EJ, Batt RM. Development of wheat-sensitive enteropathy in Irish Setters: Morphologic changes. *Am J Vet Res* 1990;51:978–982.
  80. Garden OA, Pidduck H, Lakhani KH, et al. Inheritance of gluten-sensitive enteropathy in Irish Setters. *Am J Vet Res* 2000;61:462–468.
  81. Polvi A, Garden OA, Houlston RS, et al. Genetic susceptibility to gluten sensitive enteropathy in Irish Setter dogs is not linked to the major histocompatibility complex. *Tissue Antigens* 1998;52:543–549.
  82. Elwood CM, Moore PF, Batt RM. Expression of gluten sensitive enteropathy (GSE) of Irish Setter dogs may be age dependent. *J Vet Intern Med* 2001;15:274.
  83. Hall EJ, Batt RM. Abnormal permeability precedes the development of a gluten sensitive enteropathy in Irish Setter dogs. *Gut* 1991;32:749–753.
  84. Elwood CM, Garden OA, Manners HK, et al. Altered populations of T-lymphocytes in the jejunal mucosa of Irish Setter dogs with gluten sensitive enteropathy. *Gastroenterology* 1996;110:A903.
  85. Elwood CM, Garden OA, Hamblin AS, Batt RM. Gluten challenge in Irish Setters with gluten sensitive enteropathy (GSE): Peripheral leucocyte responses. Proceedings of the European Society for Veterinary Internal Medicine Congress, Lyon, France, 1997:157.
  86. Elwood CM, Garden OA, Hamblin AS, et al. Mucosal and systemic cell mediated immune responses to gluten in Irish Setters with gluten sensitive enteropathy. *Gastroenterology* 1998;114:G3985.
  87. Hall EJ, Carter SD, Barnes A, Batt RM. Immune responses to dietary antigens in gluten-sensitive enteropathy of Irish Setters. *Res Vet Sci* 1992;53:293–299.
  88. Hall EJ, Simpson KW. Diseases of the small intestine. In: Et-

- tinger SJ, Feldman EC, eds. Textbook of Veterinary Internal Medicine, 5th ed. Philadelphia, PA: WB Saunders; 2000:1182–1237.
89. Batt RM, Needham JR, Carter MW. Bacterial overgrowth associated with a naturally occurring enteropathy in the German Shepherd Dog. *Res Vet Sci* 1983;35:42–46.
  90. Whitbread TJ, Batt RM, Garthwaite G. Relative deficiency of serum IgA in the German Shepherd Dog: A breed abnormality. *Res Vet Sci* 1984;37:350–352.
  91. Batt RM, Barnes A, Rutgers HC, Carter SC. Relative IgA deficiency and small intestinal bacterial overgrowth in German Shepherd Dogs. *Res Vet Sci* 1991;50:106–111.
  92. Day MJ, Penhale WJ. Serum immunoglobulin A determination in normal and diseased dogs. *Res Vet Sci* 1988;45:360–363.
  93. German AJ, Hall EJ, Day MJ. Relative deficiency in IgA production by duodenal explants from German Shepherd Dogs with small intestinal disease. *Vet Immunol Immunopathol* 2000;76:25–43.
  94. Day MJ. Low IgA concentrations in the tears of German Shepherd Dogs. *Aust Vet J* 1996;74:433–434.
  95. Littler RM, Garden OA, Batt RM. Immunoglobulin concentrations in faecal extracts as a measure of canine mucosal immune function. Proceedings of the British Small Animal Veterinary Association Congress, Birmingham, UK, April 1999:278.
  96. Willard MD, Simpson RB, Delles EK, et al. Effect of dietary supplementation of fructo-oligosaccharides on small intestinal bacterial overgrowth in dogs. *Am J Vet Res* 1994;55:654–659.
  97. Willard MD, Simpson RB, Fossum W, et al. Characterization of naturally developing small intestinal bacterial overgrowth in 16 German Shepherd Dogs. *J Am Vet Med Assoc* 1994;204:1201–1206.
  98. Littler RM, Batt RM, Lloyd DH. Low mucosal IgA is associated with colonisation by gut enteropathogenic *E. coli* in the GSD. Proceedings of the British Small Animal Veterinary Association Congress, Birmingham, UK, 2000:277.
  99. Garden OA, Elwood CM, Desport M, Batt RM. In situ hybridization as a technique for the immunological investigation of canine intestine: Jejunal expression of IFN- $\gamma$  and IL-10 in Irish Setters and Beagles. *Vet Immunol Immunopathol* 1999;70:1–17.
  100. Batt RM, McLean L, Riley JE. Response of the jejunal mucosa of dogs with aerobic and anaerobic bacterial overgrowth to antibiotic therapy. *Gut* 1988;29:473–482.
  101. Brandwein SL, McCabe RP, Cong YZ, et al. Spontaneously colitic C3H/HeJBir mice demonstrate selective antibody reactivity to antigens of the enteric bacterial flora. *J Immunol* 1997;159:44–52.
  102. Amin AR, Attur MG, Thakker GD, et al. A novel mechanism of action of tetracyclines: Effects on nitric oxide synthases. *Proc Natl Acad Sci USA* 1996;93:14014–14019.
  103. Attur MG, Patel RN, Patel PD, et al. Tetracycline upregulates COX-2 expression and prostaglandin E<sub>2</sub> production independent of its effects on nitric oxide. *J Immunol* 1999;162:3160–3167.
  104. Patel RN, Attur MG, Dave MN, et al. A novel mechanism of action of chemically modified tetracyclines: Inhibition of COX-2-mediated prostaglandin E<sub>2</sub> production. *J Immunol* 1999;163:3459–3467.
  105. Batt RM, Carter MW, Peters TJ. Biochemical changes in the jejunal mucosa of dogs with a naturally occurring enteropathy associated with bacterial overgrowth. *Gut* 1984;25:816–823.
  106. Batt RM, McLean L. Comparison of the biochemical changes in the jejunal mucosa of dogs with aerobic and anaerobic bacterial overgrowth. *Gastroenterology* 1988;93:986–993.
  107. Hall EJ, Batt RM. Enhanced intestinal permeability to <sup>51</sup>Cr-labeled EDTA in dogs with small intestinal disease. *J Am Vet Med Assoc* 1990;196:91–95.
  108. Hart CA, Embaye H, Getty B, et al. Ultrastructural lesions to the canine intestinal epithelium caused by enteropathogenic *E. coli*. *J Small Anim Pract* 1990;31:591–594.
  109. Hart CA, Batt RM, Saunders JR. Diarrhoea caused by *Escherichia coli*. *Ann Trop Paediatr* 1993;13:121–131.
  110. Batt RM, Rutgers HC, Sancak AA. Enteric bacteria: Friend or foe? *J Small Anim Pract* 1996;37:261–267.
  111. Hess AD, Tutschka PJ, Santos GW. Effect of cyclosporin A on human lymphocyte responses in vitro. II. Induction of specific alloantigen unresponsiveness mediated by a nylon wool adherent suppressor cell. *J Immunol* 1981;126:961–968.
  112. Hess AD, Tutschka PJ, Santos GW. Effect of cyclosporin A on human lymphocyte responses in vitro. III. CsA inhibits the production of T lymphocyte growth factors in secondary mixed lymphocyte responses but does not inhibit the response of primed lymphocytes to TCGF. *J Immunol* 1982;128:355–359.
  113. Hess AD, Donnenberg AD, Tutschka PJ, Santos GW. Effect of cyclosporin A on human lymphocyte responses in vitro. V. Analysis of responding T lymphocyte subpopulations in primary MLR with monoclonal antibodies. *J Immunol* 1983;130:717–721.
  114. Converse PJ, Hess AD, Tutschka PJ, Santos GW. Effect of cyclosporine on the response of normal human lymphocytes to cytomegalovirus in vitro. *Infect Immun* 1983;41:1226–1233.
  115. Schreiber SL, Crabtree CR. The mechanism of action of cyclosporin A and FK506. *Immunol Today* 1992;13:136–142.
  116. Ardizzone S, Porro GB. A practical guide to the management of distal ulcerative colitis. *Drugs* 1998;55:519–542.
  117. Lichtiger S, Present DH, Kornbluth A, et al. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med* 1994;330:1841–1845.
  118. Sandborn WJ, Tremaine WJ, Schroeder KW, et al. A placebo-controlled trial of cyclosporine enemas for mildly to moderately active left-sided ulcerative colitis. *Gastroenterology* 1994;106:1429–1435.
  119. Sandborn WJ. A critical review of cyclosporin therapy in inflammatory bowel disease. *Inflamm Bowel Dis* 1995;1:48–63.
  120. Fernandez-Banares F, Bertran X, Esteve-Comas M, et al. Azathioprine is useful in maintaining long-term remission induced by intravenous cyclosporine in steroid-refractory severe ulcerative colitis. *Am J Gastroenterol* 1996;91:2498–2499.
  121. Florin THJ, Roberts RK, Watson MR, Radford-Smith GL. Treatment of steroid-refractory inflammatory bowel disease (IBD) with mycophenolate mofetil (MMF). *Aust NZ J Med* 1998;28:344–345.
  122. Neurath MF, Wänitschke R, Peters M, et al. Randomised trial of mycophenolate mofetil versus azathioprine for treatment of chronic active Crohn's disease. *Gut* 1999;44:625–628.
  123. Present DH. Is mycophenolate mofetil a new alternative in the treatment of inflammatory bowel disease? *Gut* 1999;44:592–593.
  124. Radford-Smith GL, Taylor P, Florin THJ. Mycophenolate mofetil in IBD patients. *Lancet* 1999;354:1386–1387.
  125. Orth T, Peters M, Schkaak JF, et al. Mycophenolate mofetil versus azathioprine in patients with chronic active ulcerative colitis: A 12-month pilot study. *Am J Gastroenterol* 2000;95:1201–1207.
  126. Fellermann K, Steffen M, Stein J, et al. Mycophenolate mofetil: Lack of efficacy in chronic active inflammatory bowel disease. *Aliment Pharmacol Ther* 2000;14:171–176.
  127. Moreira AL, Sampaio EP, Zmuidzinis A, et al. Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing mRNA degradation. *J Exp Med* 1993;177:1675–1680.
  128. Moller DR, Wysocka M, Greenlee BM, et al. Inhibition of IL-12 production by thalidomide. *J Immunol* 1997;159:5157–5161.
  129. Meierhofer C, Dünzendorfer S, Wiedermann CJ. Protein kinase C-dependent effects on leukocyte migration of thalidomide. *J Infect Dis* 1999;180:216–219.
  130. D'Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci USA* 1994;91:4082–4085.
  131. Ehrenpreis ED, Kane SV, Cohen LB, et al. Thalidomide therapy for patients with refractory Crohn's disease: An open-label trial. *Gastroenterology* 1999;117:1271–1277.
  132. Vasilias EA, Kam LY, Abreu-Martin MT, et al. An open-label pilot study of low-dose thalidomide in chronically active, steroid-dependent Crohn's disease. *Gastroenterology* 1999;117:1278–1287.
  133. Bessler H, Gilgal R, Djaldetti M, Zahavi I. Effects of pentoxifylline on the phagocytic activity, cAMP levels, and superoxide

anion production by monocytes and polymorphonuclear cells. *J Leukocyte Biol* 1986;40:747–754.

134. Tannenbaum CS, Hamilton TA. Lipopolysaccharide-induced gene expression in murine peritoneal macrophages is selectively suppressed by agents that elevate intracellular cAMP. *J Immunol* 1989;142:1274–1280.
135. Reimund JM, Dumont S, Muller CD, et al. In vitro effects of oxpentifylline on inflammatory cytokine release in patients with inflammatory bowel disease. *Gut* 1997;40:475–480.
136. Bauditz J, Haemling J, Ortner M, et al. Treatment with tumour necrosis factor inhibitor oxpentifylline does not improve corticosteroid dependent chronic active Crohn's disease. *Gut* 1997;40:470–474.
137. D'Haens G, Van Deventer S, Van Hogezaand R, et al. Endoscopic and histological healing with infliximab anti-tumor necrosis factor antibodies in Crohn's disease: A European multicenter trial. *Gastroenterology* 1999;116:1029–1034.
138. Baert FJ, D'Haens GR, Peeters M, et al. Tumor necrosis factor  $\alpha$  antibody (Infliximab) therapy profoundly down-regulates the inflammation in Crohn's ileocolitis. *Gastroenterology* 1999;116:22–28.
139. Stronkhorst A, Radema S, Yong SL, et al. CD4 antibody treatment in patients with active Crohn's disease: A phase 1 dose finding study. *Gut* 1997;40:320–327.
140. Lehrer SB, Horner WE, Reese G. Why are some proteins allergenic? Implications for biotechnology. *Crit Rev Food Sci Nutr* 1996;36:553–564.
141. Davis PJ, Williams SC. Protein modification by thermal processing. *Allergy* 1998;53(Suppl 46):102–105.
142. Hannah SS, Laflamme DP, Marks SL. Hydrolyzed proteins in diets for the management of food hypersensitivity or inflammatory bowel disease. Proceedings of the WSAVA/FECAVA World Congress, Amsterdam, 2000:517.
143. Horrobin DF. Medical uses of essential fatty acid (EFAs). *Vet Dermatol* 1993;4:161–166.
144. Kremer JM, Lawrence DA, Jubiz W, et al. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. Clinical and immunologic effects. *Arthritis Rheum* 1990;33:810–820.
145. Stenson WF, Cort D, Rodgers J, et al. Dietary supplementation with fish oil in ulcerative colitis. *Ann Intern Med* 1992;116:609–614.
146. O'Moran CA. Nutritional therapy in ambulatory patients. *Digest Dis Sci* 1987;32(Suppl):95–99.
147. Hawthorne AB, Daneshmend TK, Hawkey CJ, et al. Treatment of ulcerative colitis with fish oil supplementation: A prospective 12-month randomized controlled trial. *Gut* 1992;33:922–928.
148. Campieri M, Gionchetti P. Probiotics in inflammatory bowel disease: New insight to pathogenesis or a possible therapeutic alternative? *Gastroenterology* 1999;116:1246–1249.
149. Wagner RD, Warner T, Roberts L, et al. Colonization of congenitally immunodeficient mice with probiotic bacteria. *Infect Immun* 1997;65:3345–3351.
150. Hessle C, Hanson LA, Wold AE. Lactobacilli from human gastrointestinal mucosa are strong stimulators of IL-12 production. *Clin Exp Immunol* 1999;116:276–282.
151. Bircher J, Muller J, Guggenheim P. Treatment of chronic porto-systemic encephalopathy with lactulose (1-4 betagalactosidofructose). *Lancet* 1966;108:890–893.
152. Meijer HP, Welters CFM, Heineman E, et al. Effect of enteral inulin on the pouch disease activity index and on epithelial gene expression within the ileoanal pouch: A randomized, placebo-controlled crossover study. *Gastroenterology* 1998;114:G1618 (abstract).
153. Bailey JS, Blankenship LC, Cox NA. Effect of fructooligosaccharide on *Salmonella* colonization of the chicken intestine. *Poult Sci* 1991;70:2433–2438.
154. Brown DH. Applications of fructooligosaccharides in human health. In: Carey DP, Norton SA, Bolser SM, eds. *Recent Advances in Canine and Feline Nutritional Research: Proceedings of the Iams International Nutrition Symposium*, 1996:41–44.
155. Sparkes AH, Pappasouliotis K, Sunvold G, et al. Bacterial flora in the duodenum of healthy cats, and effect of dietary supplementation with fructo-oligosaccharides. *Am J Vet Res* 1998;59:431–435.
156. Videla S, Vilaseca J, Garcia-Lafuente A, et al. Dietary inulin prevents distal colitis induced by dextran sulfate sodium (DSS). *Gastroenterology* 1998;114:G4541 (abstract).
157. Madsen KL, Doyle JS, Jewell LD, et al. *Lactobacillus* species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 1999;116:1107–1114.