

A STUDY OF THE PATHOGENESIS OF INFLAMMATORY BOWEL DISEASE IN THE CATS*

Kedilerde İnflammatory Bowel Disease'in Patogenezi Hakkında Bir Çalışma

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SUMMARY

The induction of dietary hypersensitivity using selected antigen was used to establish an experimental model of feline inflammatory bowel disease (IBD). Following the introduction of novel dietary antigens macroscopic and microscopic examination of the intestinal mucosa by endoscopy, bacteriological culture of endoscopically collected small intestinal wuice and histological examination of endoscopically obtained intestinal biopsies were carried out. The numbers of intraepithelial lymphocytes (IEL) and the relative numbers of plasma cells (IgG, IgM, IgA), T cells (CD4+, CD8+, CD5+) and MHC class II+ cells in the lamina propria were counted. Villus width was also measured. Measurement of the hydrogen concentration in breath was used to diagnose small intestinal bacterial overgrowth after administration lactulose and malabsorption after administration of xylose. Intestinal permeability was determined from the percentage urinary recovery of the disaccharide, lactulose, and the monosaccharide, mannitol, after administration of these sugars as an isotonic solution.

By day 165, histologically some villi were and wider, and there was an increase in the number of neutrophils and macrophages in the lamina propria of the cats fed ovalbumin.

ÖZET

Bu çalışma ile kedilerde infammatory bowel disease (IBD)'in deneysel modeli, seçilen antijenlerin kullanılmasına bağlı olarak besinsel aşırı duyarlılık reaksiyonunun uyarılması şeklinde oluşturulmuştur. Besinlerle antijen verilip bağırsağın uyarılmasını takiben endoskopi ile bağırsak mukozasında makroskopik ve mikroskopik incelemeler yapılmış, ince bağırsak sıvısından endokkopikal yöntemle bakteri örnekleri alınmış ve intestinal biyopsi örneklerinde histolojik incelemeler gerçekleştirilmiştir. Bağırsağın lamina propria katmanında intraepitelyal lenfosit sayısı, plazma hücreleri, T hücreleri ve MHC II+ hücreleri sayıldı. Villus genişliği de ölçüldü. Solunumdaki H⁺ konsantrasyonu ölçülerek hem laktuloz uygulamasıyla ortaya çıkan ince bağırsaktaki bakteriyel üreme hem de xyloze uygulamasıyla oluşan emilim bozukluğu tespit edilmiştir. İzotonik solusyonları halinde disakkaritlerden laktuloz, monosakkaritlerden mannitol verilmiş ve bunların idrarı geçme yüzdesi belirlenerek bağırsak permeabilitesi ölçülmüştür.

165. günde ovalbuminle beslenen kedilerde bazı villilerde kısıalma ve genişleme ile beraber lamina propriya katmanında makrofaq ve nötrofil sayısında artış gözlenmiştir.

INTRODUCTION

The term IBD in cats denotes a group of chronic intestinal disorders characterized by diffuse infiltration of the mucosa by various populations of inflammatory cells, including lymphocytes, plasma cells, eosinophils and neutrophils. Although Giardia or Camylobacter infections can cause a mixed inflammatory cell infiltration in the lamina propria, inflammatory bowel disease describes a chronic disorder in which no definitive causative agent can be identified (1). Inflammatory bowel disease (IBD) is well recognized as one of the most common causes of chronic vomiting and diarrhea in cats. However, there is very little information about

the pathogenesis of IBR in this species (2). The clinical features, natural history and complications of inflammatory bowel disease are poorly defined (3).

The cause of IBD in cats is unknown but it is suspected to have an immune-mediated basis (4). Current theories for IBD are based on the suggestion that a chronic antigenic challenge occurs with the subsequent development of a cytopathic immunological response (5).

Other factors which may have a role in IBD in cats include defective immunoregulation of

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the gut associated lymphoid tissue (GALT), genetic, infectious or parasitic agents, permeability defects or dietary allergies (6-8).

It is generally thought that the pathogenesis of IBD involves hypersensitivity responses to antigens in the bowel lumen or mucosa (5).

In bacterial overgrowth in cats, the most commonly isolated anaerobes were *Bacteriodes*, *Eubacterium* and *Fusobacterium* spp., while *Pasteurella* spp. were the most common aerobes (9). Increased numbers of bacteria in the small intestine were found to be related to feline IBD (10).

MATERIALS and METHODS

Experiment 1: In the first experiment, two groups of five months old, female DSH cats were used. These animals were specific pathogen free derived, barrier maintained cats, vaccinated against feline panleucopenia and feline respiratory viruses. Group 1 was fed with a diet based on soybean flour type I (roasted, non toxic, Sigma S-9633) and group 2 received soybean flour type I (not roasted, toxic, Sigma S9758). The soybean flour was mixed with canned food and given at a daily dose of 5g for seven days.

Food was withheld overnight on day 7 and breath hydrogen test were performed on day 8th. The breath test were repeated on day 17. Blood samples were collected and examined for haematological parameters on day 8. Endoscopy was performed in order to obtain mucosal biopsies and to investigate the bacterial flora on day 11.

On day 36 the cats were rechallenged with the same amount of soybean flour for seven days. The breath test were repeated on days 43 and 47. Blood samples were collected and examined for haematological parameters on day 43. Intestinal permeability testing was performed on day 45. Endoscopy was performed in order to obtain mucosal biopsies and investigate the bacterial flora on day 47.

Intestinal functional test and endoscopy were performed on days 90 and 91 to confirm that the cats were normal. On day 92, the cats were fed

10g soybean for one day and on day 93 intestinal functional tests were carried out. On day 94, the cats were subject to endoscopy. On day 94, soybean was reintroduced at 10g/daily for 5 days and the cats were checked with intestinal functional tests on day 96 and 103. The intestinal functional test were shown to be normal on day 103, and the cats were endoscoped on day 104 of the experiment to obtain mucosal biopsies and to investigate the bacterial flora.

Experiment 2: In the second experiment a similar procedure was followed, and three groups of 5 month old female DSH cats were used. Group 1 was fed with a diet based on soybean flour type I (not roasted, Sigma S-9633), Group 2 received ovalbumin (Sigma, A-5253 Grade II) and Group 3 were control cats which received a normal diet. The cats were fed with antigen mixed with canned food and given at a daily dose of 10g for seven days.

Endoscopy was performed in order to obtain mucosal biopsies and to investigate the bacterial flora on day 0 and repeated on day 7 of the experiment. Breath hydrogen test were performed on day 9 and 10. The breath test were repeated on days 13, 20, and 28. Intestinal permeability test were performed on day 11.

The intestinal functional test were shown to be normal on day 28, so the cats were rechallenged on day 32 with the same amount of soybean flour and ovalbumin for seven days. Breath hydrogen test were performed on day 39. Intestinal permeability test were performed on day 40. The cats were endoscoped on day 41 of the experiment to obtain mucosal biopsies and to investigate the bacterial flora.

The intestinal functional test were shown to have returned to normal on day 135, and the cats were rechallenged on day 157 with the same amount of soybean flour and ovalbumin for seven days. Breath hydrogen test were performed on day 163 and again on day 170. Intestinal permeability tests were performed on day 164. The cats were endoscoped on day 165 of the experiment to obtain mucosal biopsies and to investigate the bacterial flora.

Breath Hydrogen Tests: Breath hydrogen tests were carried out according to the

procedure described by Muir (10) and Pappasoulotis (11).

A breath hydrogen xylose tests was considered abnormal if the breath hydrogen increase over the baseline level (at time 0) equalled or exceeded the mean plus twice the standard deviation was that the increase in hydrogen excretion was sustained for at least three consecutive time points (45 minutes) establish previously in normal cats using this method (11). Two criteria for identifying cats with abnormal breath hydrogen lactulose tests were used (11).

1. The mean value the exhaled hydrogen after 15 minutes plus the mean value of the exhaled hydrogen after 30 minutes were added and the arithmetic mean was calculated. Results were compared with compared with the range determined previously in healthy cats.

2. As the second criterion, the four values of hydrogen concentration in the first 45 minutes were added together to provide an approximation to the area under the curve. The mean value of the area under the curve was calculated and was considered abnormal if it exceeded the mean plus twice standard deviation previously determined in healthy cats. A breath hydrogen lactulose test was considered abnormal only if both criteria were fulfilled.

Lactulose-Mannitol Intestinal Permeability Test: Lactulose-mannitol tests were carried out according to the procedure described by Pappasoulotis (11).

Results of recovery ratios of lactulose and mannitol were compared with a reference range calculated for healthy cats described previously by Pappasoulotis (8) using the same method. The lactulose mannitol recovery ratios range were 0.05-0.83.

Endoscopy: Upper gastrointestinal endoscopy was performed under general anaesthesia, using a flexible fiberoptic gastroscope with a 3.5 and 4 mm diameter insertion tube (Olympus gastrointestinal fibrescope P3 with a 100 C forward viewing lens Keymed). Food was withheld from cats overnight. The cats were anaesthetised and placed in left lateral recumbancy. Before endoscopy the instrument was disinfected with a

preparation containing formalin and succindialdehyde (Gigasept, Sterling Medicare). The instument channel was flushed with sterile saline and then air. Small intestinal mucosal biopsies were collected by endoscopy. Five biopsies approximately 2 mm³ were taken from the duodenum and formalin-fixed.

Bacteriology: A sample of small intestinal fluid was collected by endoscopy. The aspirate (100µl) was taken from the duodenum using a sterile 1.8 mm diameter catheter and a 10 ml syringe. The sample was placed in a sterile vacutainer. The air in the vacutainer was removed using a syringe to protect oxygen sensitive bacteria. After sampling, dilutions of the specimen were put into an anaerobic cabinet (Whitley Anaerobic Cabinet Mk III, Don Whitley Scientific Limited, England) and inoculated onto agar plates within 10 minutes of sampling. Media used for bacterial culture were blood agar, Mac Conkey agar, FAA (Fastidious anaerobe agar) with and without gentamycin. A series of dilutions of the specimen from 10¹ - 10⁴ in sterile saline were prepared to detect anaerobic and aerobic bacteria. Plates were prepared for the detection of anaerobes and aerobes. Plates for anaerobes were inoculated and incubated in an anaerobic cabinet, and for aerobes were inoculated in room air and incubated in 5 % CO₂ atmosphere (Campyak; BLL Microbiology System). A drop (10µl) of each dilution was spread on plates using a sterile loop. After appropriate incubation, colonies on the plates were counted and the species identified using standard techniques.

The results of bacteriological investigation were compared with these previously described by Pappasoulotis (11) for healthy cats using the same methods. Total bacterial counts higher than 10⁷ cfu/ml and anaerobic and aerobic counts ≥ 10⁶ cfu/ml were considered abnormal.

Morphometric studies: Small intestinal mucosal biopsies were collected by endoscopy. Five biopsies approximately 22 mm³ were taken from the duodenum and formalin-fixed. Sections were cut at 4µm and stained with haematoxylin and eosin (H&E). Villus width were measured and the number of intraepithelial lymphocytes (IEL) were counted and expressed as numbers of IEL/100 enterocytes using video

assisted microscopy (VIDS, Image Processing Systems, Synoptic Ltd, England).

Immunostaining Techniques: Characterisation of small intestinal lymphoid cells was performed by immunohistochemistry. The number of IgA, IgG, and IgM producing plasma cells, CD4+, CD5+ and CD8+ cells was examined in small intestine from frozen tissue and formalin fixed tissue by using standard immunoperoxidase tests.

The positively stained mononuclear cells were counted by VIDS as cells/mm² in a particular area of tissue.

RESULTS

Two separate experiments were conducted, as described in materials and methods. In these experiments, the number of cats were too few for meaningful statistical analysis.

In the first experiment, xylose breath testing was normal on all days in the group given roasted soybean with the exception of one of the two cats which had an abnormal test on day 93. Lactulose breath testing was abnormal on days 17, 43, 93 and 96 for cats fed roasted soybean and was abnormal on days 43, 47, 90, 93, 96 and 103 for both cats fed un-roasted soya.

The mean villus width for cat B fed roasted soybean was higher on days 47, 92 and 94 when compared with day 11. Mean villus width for cat A fed roasted soybean was higher on day 92 compared with days 11 and 94. Mean villus width for cat C fed un-roasted soya was higher on day 47 compared with days 11 and 92. On day 92 the villus width for cat D fed un-roasted soybean was increased on day 92 compared with days 11 and 47. The mean IEL count gradually increased during the experiment for the cats given roasted soybean. The mean IEL count for cat C fed un-roasted soybean was higher on day 11 compared with days 47 and 92. The mean IEL count for cat D fed un-roasted soybean was higher on day 47 compared with days 11 and 92.

The number of anaerobic bacteria was increased on day 47 compared with day 11 in both experimental groups, but remained relatively consistent on days 92 and 94 in cats fed

un-roasted soybean. The numbers of total and aerobic bacteria cultured from the intestine of cat A fed roasted soybean were found to be decreased on days 92 and 94 compared with day 11. In contrast, the samples from the intestine of cat B fed with the same protein were found to have increased numbers of bacteria on days 47, 92 and 94 compared with day 11.

The numbers of total and aerobic bacteria cultured from intestine of cat C fed un-roasted soybean were found to be increased on days 47 and 92, but decreased on day 94 compared with day 11. However, the samples from the intestine of cat D remained consistent on days 11, 47 and 92, but decreased on day 94. On day 11 *Escherichia coli*, *Pseudomonas paucimobilis* and *Clostridium syy.* were isolated from all cats in both groups. On day 47 *Clostridium spp.*, faecal *Streptococci*, *Pasteurellae* and *Diphtheroid spp.* were isolated from cats B-D. Non-haemolytic *Streptococcus spp.* were isolated on day 94 from cats in both experimental groups.

The percentage of urinary lactulose and mannitol was lower in both groups on day 45 compared with day 10. The La/Ma ratio was higher on day compared with day 10 for cats A and C. Urinary mannitol was not detected by the enzymatic assay for cat B on day 45, which may reflect the digestion of mannitol by the increased number of bacteria in the gut.

In the second experiment, xylose breath testing was normal for control animals on all occasions but was abnormal for both cats given ovalbumin on days 20, 28, 39 and 170. Xylose breath testing was also abnormal for both of the two cats receiving roasted soybean on days 13, 20 and 39. Lactulose breath testing was abnormal on days 9, 13, 20, 28, 39 and day 170 for both groups. Lactulose breath testing was normal for control cats on each occasion.

The numbers of aerobic bacteria cultured from the intestine of cats fed ovalbumin were found to be decreased on days 7 and 165 compared with day 0. The numbers of aerobic bacteria cultured from the cats fed roasted soybean were found to be increased on day 7 compared with days 0 and 41. The numbers of anaerobic bacteria cultured from the cats fed ovalbumin were found to be increased on day 7 and 41,

but to be decreased on day 165. The total numbers of bacteria for cats fed ovalbumin on day 0 were similar on days 7 and 41, but lower on day 165. The total numbers of bacteria and anaerobic bacteria for cats fed ovalbumin were lower on day 165 compared with days 0, 7 and 41 of the experiment. The total numbers of bacteria for cats given roasted soybean on day 0 were similar to days 7, 41 and 165. The numbers of the aerobic, anaerobic and total bacteria on days 7 and 41 were similar for control cats.

Clostridium, *Bacteroides*, *Pasteurella*, *Streptococcus* spp., *Escherichia coli* and other coliforms were observed in the cultures of duodenal aspirates from the cats in experiment 2 on days 0, 7, 41, 165. *Streptococci*, coliforms, *Pasteurella* and *Clostridium* spp. were isolated in the cultures of duodenal aspirates from cat A and B on day 0 of the experiment. Coliforms, *Streptococci* and *Bacterioides* spp. were isolated from control cats fed a normal diet only days 7 and 41. On day 7 coliforms, clostridia and Gram negative *Bacillus* spp. were isolated from both groups of experimental cats. On day 41 non-haemolytic *Streptococci* and faecal *Streptococci* spp. were isolated from cats fed OVA and roasted soybean. *Neisseria* spp. were isolated from cats fed OVA only. Finally, on day 165 faecal *Streptococci* and *Pasteurellae* spp. were isolated from cats fed OVA and roasted soybean. In addition *Staphylococcus epidermidis*, *Pasteurella*, coliforms and *Bacteroides* spp., were isolated from cats fed soybean only.

Throughout the second experiment, an increasing number of IEL was observed for cats ovalbumin or roasted soybean from day 0 to day 165, in contrast to the control cats where the number remained consistent on days 7 and 41. In cats fed ovalbumin, the numbers of IEL were found to be higher when compared with cats fed roasted soybean on day 165. The numbers of IEL were similar in both experimental groups on day 0, 7, and 41. The measurement of villus width increased in cats fed ovalbumin and roasted soybean on days 7, 41 and 163 compared with day 0.

The numbers of immunoglobulin bearing plasma cells were counted in the lamina propria of biopsies from cats in experiment 2 following immunohistochemistry. The number of plasma

cells of each class increased in both experimental groups compared with day 0 after every rechallenge. The number of plasma cells remained consistent in control cats on day 7 and 41.

The numbers of T cells bearing the CD4+, CD5+ or CD8+ molecules were also determined in the lamina propria and epithelium of cats in experiment 2. The number of each cell type remained consistent in control animals on days 7 and 41. The numbers of T cells bearing the CD4+, CD5+ or CD8+ molecules increased in both experimental groups compared with day 0, and this increase occurred gradually after every rechallenge.

Small intestinal biopsies were collected from the duodenum by endoscopy and formalin-fixed and paraffin-wax embedded for histopathological assessment. Biopsies were taken on days 0, 7, 41 and 165. There was no significant histopathological abnormality detected in biopsies taken on days 0, 7, and 41 however by day 165 changes were observed in the cats fed ovalbumin. At this time, some villi were shorter and wider, and there was an increase in the number of neutrophils and macrophages in the lamina propria of both cats fed ovalbumin (Figure 1). Biopsies taken at these time were also stained for T and B cell markers as described previously. At day 165 in the ovalbumin fed group, there were numerous CD4+, CD5+ and CD8+ T cells in the lamina propria at the site of the pyogranulomatous inflammation.

The percentages of lactulose and mannitol in the urine of cats fed ovalbumin, and of lactulose in the urine of cats fed roasted soybean, were higher on day 11 compared with days 40 and 164. The La/Ma ratio was found to be very high on day 40 compared with days 11 and 165 for cat C. Mannitol was not detected by the enzymatic assay for Cat D on day 165, which may reflect digestion of mannitol by increased numbers of intestinal bacteria at this time.

DISCUSSION

Current theories IBD in the cat are based on the suggestion that an antigenic challenge occurs with the subsequent development of a cytopathic immunological response (5). Some

researchers have suggested that dietary antigens may be an important factor in canine and feline IBD (12).

The model of IBD developed in the present study showed some similarities to spontaneously occurring feline IBD, such as an association with bacterial overgrowth and abnormal intestinal functional tests, but differed histopathologically. Spontaneously occurring feline IBD is most often characterized by excessive numbers of lymphocytes and plasma cells in the gastrointestinal tract (13). In the model of feline inflammatory bowel disease described here, the inflammatory infiltrate consisted primarily of neutrophils and macrophages. This model may be different from naturally occurring feline IBD, but it is also possible that some predisposing factors other than dietary sensitisation may be influential on the differential occurrence of these forms of feline IBD.

Determination of villus morphology and epithelial integrity was carried out in the experimental cats. However, the use of pinch biopsies has limitations, since only small biopsies are obtained which may limit histological morphometric measurement due to orientation of the villi in processing. Linear micrometer measurements of pinch biopsies of feline mucosa suffers from the same limitations as quantitative studies in dogs (14). Therefore, the measurements of villus height and crypt depths were not made, because the decision as to where a villus ends and a crypt begins was often arbitrary.

Increased IEL numbers have been reported in coeliac disease in man, and in children with cows milk intolerance (15,16). High IEL have also been recorded in *Giardia* and *Hexamita* infections (17,18). During the present study IEL counts were found to be significantly higher in cats challenged with OVA and soybean when compared with controls in experiment 2. In experiment 1, even though there was no obvious histopathological changes, some changes were recorded including an increased number of intra-epithelial lymphocytes.

In both experiments there was also an increase in the number of plasma cells within the lamina propria following challenge of sensitized

animals. Intestinal mucosal cell populations in cats might therefore, be influenced by dietary proteins. In experiment 2, the numbers of IgA, IgG and IgM-containing cells were increased in the lamina propria after rechallenging with soya or ovalbumin (19). In most studies of human IBD, an increase in the number of plasma cells bearing IgA and IgG has been found (20,21). The local response pattern of Ig-containing cells was compared in Crohn's disease and ulcerative colitis by immunohistochemistry of large bowel wall, and the numbers of IgA, IgG and IgM bearing cells increased more than three times compared with controls (22). In the model of feline inflammatory bowel disease described here, marked increases in IgA, IgG and IgM producing cells in the intestinal mucosa may be associated with local immunoglobulin secretion. The regulation of the local mucosal response to enteric bacteria or luminal foreign material may be broken after encountering dietary antigens. Consequently, immunoglobulin bearing plasma cells may be activated. Such responses may be mediated by activation of immune cells in the mucosa causing release of chemical mediators that act directly or indirectly on the epithelium. Damage to the intestinal mucosa may then allow more of the initiating antigens to gain access to the mucosa, thus perpetuating the lesion (12). In contrast to these results, Strombeck (1) observed that mucosal IgA secretion was typically decreased in IBD leishinosis. An increased number of IgA and IgG bearing plasma cells in the lamina propria is not a stable characteristic in the immunohistological assessment of canine inflammatory bowel disease (23).

The number of CD4⁺ and CD8⁺ T lymphocytes within the intestinal mucosa significantly increased after challenge with dietary antigen. A similar increase was also reported for CD4⁺ and CD8⁺ T lymphocytes in both Crohn's disease and ulcerative colitis in man (24-26). The majority of infiltrating T lymphocytes in both Crohn's disease and ulcerative colitis were activated CD4⁺ helper cells (27). Jergens and colleagues (23) have shown that there is a significant number of T cells in the lamina propria of dogs with IBD.

The previous dietary history of these cats was known and had not included soybean or

ovalbumin. Mucosal biopsy specimens were obtained endoscopically after every challenge to evaluate mucosal epithelial damage and the presence of any inflammatory infiltrate. In experiment 1, although histopathological changes consistent with IBD were not observed, there was an increased number of anaerobic bacteria, villus width and positive breath hydrogen lactulose test results after each rechallenge of the cats. In experiment 2, in addition to functional changes, histological abnormalities were demonstrated on day 165 after challenging the cats three times with ovalbumin, but not with soybean. It is possible that these differences between antigens may be related to their relative allergenicity or toxicity. The findings in these studies suggest that ovalbumin may be a useful dietary antigen for study of the pathogenesis of IBD. The dosage of ovalbumin was higher in experiment 2, and it may be that the dosage of dietary antigen, as well as age breed and sex of the cats is important in the development of IBD. For these reasons the experimental protocols used here should be further refined in the future.

Clinical signs of intestinal disease did not appear in the cats in these experiments, despite abnormal gut histology and abnormal breath hydrogen lactulose tests.

Intestinal permeability is the property of the intestinal epithelium that allows certain molecules to be absorbed through the mucosa passively, without the assistance of a biochemical carrier system (28). Investigation of intestinal permeability is important for understanding the pathophysiological mechanisms of intestinal disease and for the diagnosis of intestinal mucosal damage. In the case of gut damage, there is reduced mucosal surface area and villus atrophy, leading to decreased absorption of small molecules (monosaccharides), whilst disruption of the tight junctions leads to increased permeation of large molecules (disaccharides). The ratio of the disaccharide-to-monosaccharide urinary recovery can eliminate the effect of irrelevant variables which influence a single test such as intestinal dilution, small intestinal transit time, renal excretion, bacterial degradation of probes and incomplete urine collection (11,28).

In experiment 2, although abnormal La/Ma ratio changes in urine were not observed, a rel-

ative increase in the permeation of lactulose was seen after the first sensitization compared with second and third sensitizations. This suggests that there may have been some damage to tight junctions. This a well established indicator of intestinal damage in man (29-31) and abnormalities have also been reported in dogs with gluten hypersensitivity using a cellobiose and mannitol permeability test (32).

Increases in permeation may be an initiating factor in the development of intestinal disease by allowing increased permeation of macromolecules leading the hypersensitivity or may simply be a consequence of intestinal damage (29-31).

Breath hydrogen levels after administration of a source of readily absorbable carbohydrate such as xylose reflect the absorptive function of the small intestine but can also be influenced by bacterial overgrowth in the small intestine leading to early fermentation. Measurement of hydrogen after administration a test meal consisting of a non-digestible carbohydrate such as lactulose provides information about intestinal transit time through indicating arrival of the test meal in the colon, but can also be influenced by small intestinal bacterial overgrowth leading to earlier hydrogen production (10,11,33). The abnormal breath hydrogen lactulose tests after each challenge suggested that bacterial overgrowth occurred in experiment 2. Results of the xylose breath tests did not show clear evidence of malabsorption or bacterial overgrowth, even when gut inflammation occurred on day 165 of experiment 2. It is possible that the breath lactulose test is more sensitive than the breath hydrogen xylose test in identifying bacterial overgrowth or that these may have been to false positives.

There was poor correlation in the evidence of small intestinal bacterial overgrowth based on the results of breath hydrogen testing and bacteriological examination of small intestinal aspirates obtained endoscopically. Although small intestinal bacteriology is often considered the definitive test for detecting bacterial overgrowth both procedures have their advantages and disadvantages. The absence of fermenting bacterial flora may lead to a negative breath test in a cat with bacterial overgrowth (34).

Some workers suggest that identification of hydrogen production in the small intestine does not necessarily indicate bacterial overgrowth (35) whereas other researchers suggest that hydrogen is produced in the small intestine only in case of bacterial overgrowth (36). The presence of a relatively high number of bacteria in the small intestine in normal individuals (9) may be of relevance in cats. Contamination with oral flora may also lead to a false positive breath test in diagnosing bacterial overgrowth. Therefore any delays between sampling and inoculation onto culture media and inadequate bacteriological technique may be limiting factors (37). It has been suggested that mucosal biopsies may be more reliable for diagnosing bacterial overgrowth by culture than intestinal aspirates reflecting adherence of the bacteria to the mucosal surface. However comparative studies of the two techniques have failed to show any advantages of using mucosal biopsies (12).

Metabolic interactions between the intestinal microflora and the host have also been suggested to play a role in the pathogenesis of chronic IBD. High protein or fat diets in patients with Crohn's disease or ulcerative colitis are reported to decrease anaerobic bacteria, and to change the composition of the intestinal microflora (38).

The number of intestinal bacteria in the cats which had not been exposed to a novel dietary antigen on day 0 was lower than in the proximal small intestine of normal cats reported previously by Johnston and colleagues (9) but higher than in the normal proximal small intestine of man (39), or in the clinically healthy cats reported by Muir and colleagues (10). After rechallenging the cats in the present experiments by novel dietary proteins, bacterial overgrowth was observed when the results were compared with control cats and clinically healthy cats (9).

CONCLUSION

In summary, the development of the model of feline IBD described here, indicates that food allergy, may possibly be involved in the aetiology of IBD in cats.

Further experimental studies into food hyper-

sensitivity in cats may provide additional evidence regarding the aetiology of feline IBD.

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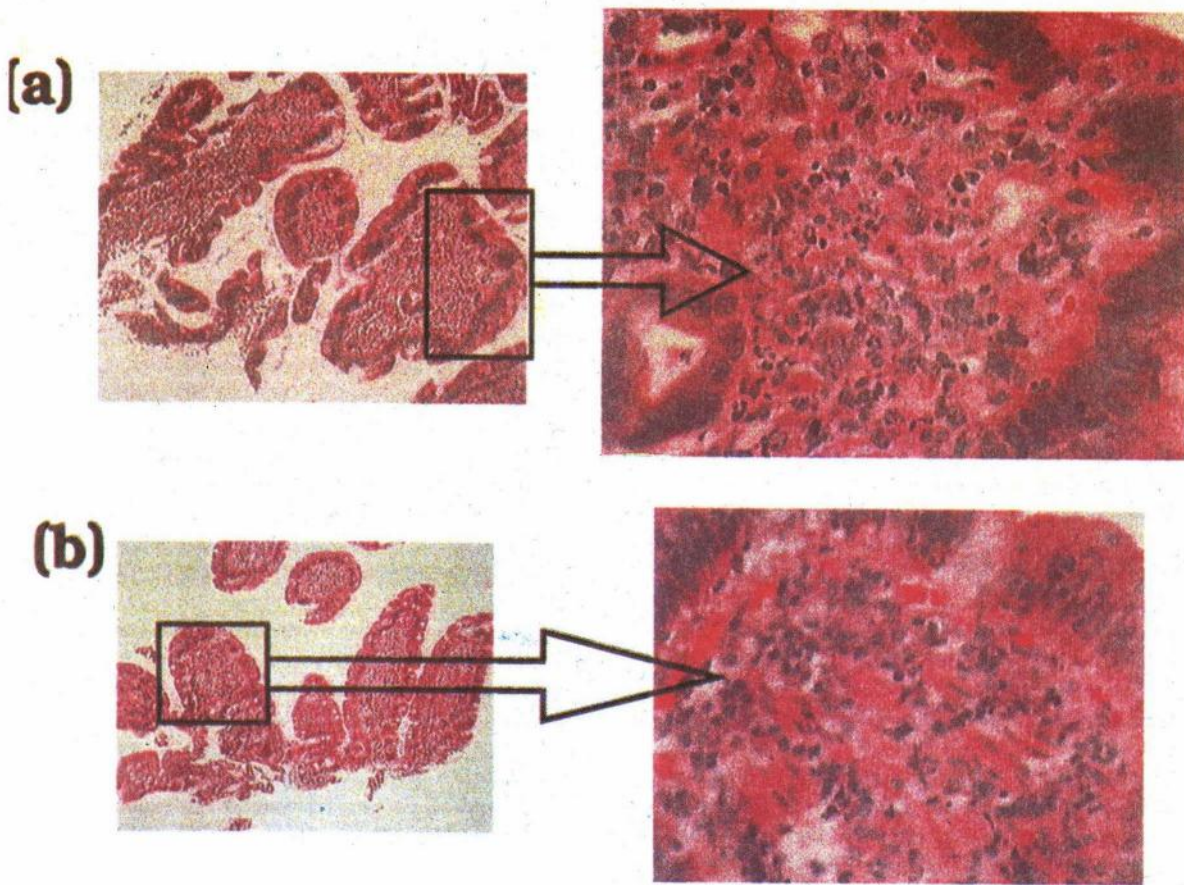


Figure 1. Duodenal biopsies taken from two cats (a b) sensitized and challenged with ovalbumin. Biopsies were taken on day 165 of experiment II. Sections were fixed and stained with H E. Lower power fields (x25) demonstrate some villus stunting and an infiltration of mononuclear cells. On high power (x100) these were found to be predominantly macrophages and neutrophils.