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BACTERIAL FLORA AND INTESTINAL CHRONIC DISEASES
FLORA BATTERICA E MALATTIE CRONICHE INTESINALI

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THE PRECURSORS OF FECAPENTAENES. A PRELIMINARY REPORT
I PRECURSORI DEI FECAPENTENI. RAPPORTO PRELIMINARE
BACTERIAL FLORA AND INTESTINAL CHRONIC DISEASES

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Summary. - There are three conditions to be considered, small intestinal bacterial overgrowth, Crohn’s disease (CD) and ulcerative colitis (UC). Alterations in the luminal flora occur on these conditions, but little is known about the mucosal-associated bacteria. Surgical mucosal samples were obtained from the jejunum, ileum, colon and rectum from control subjects and patients with Crohn’s disease. The mucosal associated flora of the normal small intestine and colon yielded $10^4$ and $10^6$ organisms/g tissue respectively, whereas Crohn’s tissue yielded $10^6$ org/g in the ileum and colon. The bacteria isolated were qualitatively similar in both groups and the ratio of anaerobes : aerobes was 1 : 1. The distribution of the colonic mucosal flora was studied by culturing multiple endoscopic biopsy samples obtained from rectum to ileum from each patient. A ratio of anaerobes : aerobes was 1 : 1 with Bacteroides species and Enterobacteriaceae as the commonest organisms isolated. Experimental studies on inflammatory bowel disease have suggested the involvement of Enterobacteriaceal common antigen, Mycobacterium paratuberculosis and Bacteroides vulgatus in the pathogenesis of CD and experimental UC. Results of serum antibody titres to antigens derived from these strains are discussed.

Riassunto (Flora batterica e malattie croniche intestinali). - Ci sono 3 condizioni da considerare: la sovraccrescita batterica nel piccolo intestino, il morbo di Crohn e la colite ulcerosa. In queste condizioni si riscontrano alterazioni della flora luminale ma poco si sa sui batteri associati alla mucosa. Campioni di mucosa sono stati prelevati chirurgicamente dal digiuno, dall'ileo, dal colon e dal retto da soggetti di controllo e da pazienti affetti da morbo di Crohn. La flora associata alla mucosa dell'intestino tenue e del colon normali hanno dato luogo alla crescita rispettivamente di $10^4$ e $10^6$ organismi/g di tessuto, mentre tessuti provenienti da pazienti affetti da morbo di Crohn hanno dato luogo alla crescita di $10^6$ organismi/g nell'ileo e nel colon. I batteri isolati erano qualitativamente simili in entrambi i gruppi e il rapporto anaerobi: aerobi era di 1 : 1. La distribuzione della flora mucosale del colon è stata studiata coltivando multipli campioni bioptici endoscopici prelevati dal retto all'ileo di ciascun paziente. Il rapporto anaerobi: aerobi era di 1 : 1 essendo i Bacteroides sp. e le Enterobacteriaceae gli organismi isolati più comunemente. Studi sperimentali sulle malattie croniche intestinali hanno suggerito un coinvolgimento di un antigene comune delle Enterobacteriaceae, del Mycobacterium protubercolosis e del Bacteroides vulgatus nella patogenesi del morbo di Crohn e della colite ulcerosa sperimentale. Vengono discussi i risultati dei titoli anticorpali sierici agli antigeni derivati da questi ceppi.
Introduction

The intestinal flora of the gastrointestinal tract is derived from the oropharynx, saliva and food. It consists of two types of flora, lumenal and mucosa-associated. The normal indigenous microflora varies very little under constant conditions unless the integrity of the intestine is deranged. Under abnormal conditions intestinal flora may contribute directly to malabsorption and nutritional deficiencies in the host in conditions associated with small intestinal bacterial overgrowth, and the microflora may contribute to the pathogenesis of chronic inflammatory disease.

In this paper we shall consider three main conditions, firstly, small intestinal bacterial overgrowth, secondly Crohn's disease and finally ulcerative colitis.

Small intestinal bacterial overgrowth.

Three types of gastrointestinal abnormality encourage bacterial growth in the small intestine. Since gastric acidity is important in controlling the entry of microorganisms into the small intestine the first group includes abnormalities of the stomach such as gastric atrophy due to Addisionian pernicious anaemia, partial gastrectomy and vagotomy with pyloroplasty or gastroenterostomy.

Secondly, there may be areas of stasis within the small intestine as in diverticulosis of duodenum and jejunum, or surgically created blind loops due to enterointerostomy and strictures. If normal peristalsis is inhibited, as in scleroderma, diabetic neuropathy or after treatment with ganglion-blocking agents, a profuse bacterial flora may also develop in the areas of stasis.

It has been further claimed that bacterial overgrowth may occur in the small intestine in old age without demonstrable intestinal lesions (1). Whether this is due to an undiscovered and occult lesion, is at present uncertain.

Thirdly, there may be a free communication between the small intestine and colon through a fistula, as in gastrocolic or enterocolic fistulae, or following surgical anastomoses between the large and small bowel. Under these circumstances, the small intestine is exposed to the high concentration of bacteria in the colon, which have unimpeded entry as a result of the higher pressure present in the colon than in the small intestine. This may also occur after resection of the small intestine with the loss of the ileocaecal valve.

(i) Bacterial flora. - The development of an abnormal intestinal flora is related to the site and extent of the causative abnormality (2). In a patient with multiple jejunal diverticulosis, for example, there may be a diffuse and generalized bacterial proliferation throughout the small intestine, and the same situation may develop in a generalized disorder of motility, such as scleroderma. On the other hand, a single duodenal diverticulum may be associated with a significant bacterial flora which is limited to the upper intestine, and conversely a distal intestinal stricture, involving the terminal ileum, for example, may encourage bacterial proliferation only in the ileum.

The bacterial counts in intestinal fluid from patients with conditions favouring stasis are usually markedly increased, ranging from $10^6$ to as high as $10^9$ organisms/ml. Coliforms and other aerobic organisms are invariably present, but there are also high counts of anaerobic organisms such as bacteroides, anaerobic lactobacilli and clostridia. These anaerobic organisms are of particular importance since it is the anaerobic flora that is responsible for the majority of the deleterious effects of bacteria in the small intestine.

The nature of the abnormal bacterial flora may be determined by the presence or absence of stasis, and the development of the anaerobic flora is particularly correlated with areas of stasis. As in the case after small intestinal resection, the site and extent of the causative lesion may therefore be of great importance in determining the severity of malabsorption and malnutrition that may result.
Metabolic and clinical consequences of small intestinal bacterial overgrowth. - The type of metabolic activities occurring in the small intestine and their clinical consequences will depend on the type of organisms and their concentrations within the lumen of the small bowel.

High concentrations of bacteria in the small intestine will interfere with normal bile acid metabolism leading to bile salt deconjugation and steatorrhoea in these patients. Bacteria will also cause malabsorption of vitamin B12 and of other fat-soluble vitamins and interfere with carbohydrate and amino-acid metabolism. If the malabsorption is severe then it can lead to nutritional deficiencies in the host such as megaloblastic anaemia and kwashiorkor. Extensive reviews of this subject are available elsewhere (3, 4).

Mucosa-associated flora. - Previous bacteriological studies of the intestine of patients with Crohn's disease have concentrated on examining the contents of the intestinal lumen. These studies have revealed abnormalities in small bowel flora caused by the pathological changes such as strictures and fistulae, which predispose to stasis and bacterial overgrowth (2, 5, 6).

Bacteria however do not exist solely in the lumen; in experimental animals a specific bacterial flora intimately associated with the intestinal epithelium has been demonstrated (7, 8), and there is a possibility that a mucosal flora exist in man (9, 10). Furthermore, intramural bacteria have been reported in Crohn's disease (11) and symptomatic improvement has been observed in patients treated with antibacterial drugs (12). This has led us to investigate the mucosal flora in patients with Crohn's disease. The bacterial flora intimately associated with the intestinal mucosa of patients with Crohn's disease, including both histologically involved and uninvolved tissue compared with that of patients in a control group who were not suffering from inflammatory bowel disease were investigated (13). Whole thickness intestinal sections were taken from surgical specimens. A section of each specimen was examined histologically. Twenty two specimens of Crohn's tissue (12 ileum, 10 colon) and 46 control samples from small and large bowel were examined using a strictly anaerobic bacteriological technique in an anaerobic chamber. Organisms were grown under both aerobic and anaerobic conditions. A mucosal flora was found to exist in all the large bowel samples and in three-quarters of the small bowel samples. It was qualitatively similar in all samples, consisting mainly of Gram positive bacteria, aerobic Gram negative rods and bacteroides. Greater numbers of bacteria were associated with colonic tissue ($10^7$-$10^8$ per g) than with tissue from the jejunum ($10^3$-$10^4$ per g). Samples from the terminal ileum were quantitatively intermediate between jejunum and colon. There was no statistical difference in the numbers of bacteria associated with Crohn's tissue compared with histologically normal tissue from the same patients and from the control group of patients (Table 1). Among the bacterial isolates, however, Enterobacteria were more commonly associated with Crohn's disease tissue (Table 2). Gram positive anaerobic bacteria were also isolated although bacteroides were the commonest anaerobic bacteria (Table 3). No mycobacteria were isolated from any of the samples using the conventional culture techniques for mycobacteria.

The pathogenesis of inflammatory bowel disease (IBD). - Discovery of the causes of Crohn's disease and ulcerative colitis remains the ultimate goal of workers interested in inflammatory bowel disease. Until an aetiologcal agent is discovered, therapy must inevitably be directed towards symptomatic relief. A number of the different hypotheses have been investigated. Amongst these are the potential infectious agents, the transmissible agent in Crohn's disease.

The involvement of immunological mechanisms in the pathogenesis of inflammatory bowel disease (IBD) has been extensively investigated (14-17). Broberger and Perlmann (15) demonstrated the presence of an antibody in the sera of patients with ulcerative colitis which reacted with an antigen derived from human colon. The possibility that antigens derived from intestinal bacterial products may cause this immune stimulation led to the consideration that the intestinal flora may play a pathogenic role, particularly *Escherichia coli* 014.
Table 1. - Mucosal flora in Crohn's disease. Log_{10} no. Bacteria/gram tissue (range)

<table>
<thead>
<tr>
<th>Site</th>
<th>Controls</th>
<th>Crohn's disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N. specimens (positive cultures)</td>
<td>Mean total anaerobes</td>
</tr>
<tr>
<td>Jejunum</td>
<td>4(3)</td>
<td>4.1(3.7-4.3)</td>
</tr>
<tr>
<td>Ileum</td>
<td>10(9)</td>
<td>3.8(2.8-5.5)</td>
</tr>
<tr>
<td>Colon</td>
<td>6(6)</td>
<td>6.1(3.1-6.3)</td>
</tr>
<tr>
<td>Rectum</td>
<td>2(2)</td>
<td>6.1(6.0-6.1)</td>
</tr>
</tbody>
</table>

NC = No cultures.

Table 2. - Facultative bacteria isolated

<table>
<thead>
<tr>
<th>Tissue</th>
<th>N. facultative isolates</th>
<th>Percentage of isolates of stated group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gram-negative rods</td>
</tr>
<tr>
<td>Normal</td>
<td>142</td>
<td>44.5</td>
</tr>
<tr>
<td>Crohn's</td>
<td>117</td>
<td>58.5</td>
</tr>
</tbody>
</table>

Table 3. - Strict anaerobes isolated

<table>
<thead>
<tr>
<th>Tissue</th>
<th>N. anaerobic isolates</th>
<th>Percentage of isolates of stated group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bifidobacteria</td>
</tr>
<tr>
<td>Normal</td>
<td>166</td>
<td>64.0</td>
</tr>
<tr>
<td>Crohn's</td>
<td>43</td>
<td>61.0</td>
</tr>
</tbody>
</table>

Escherichia coli 014 was found to have a common antigen which reacted with human colonic mucosa (16, 18, 19) and autoantibodies to gut mucosa could be produced by injection of bacteria (20). Further work demonstrated increased incidence of antibodies to Escherichia coli 014 in the serum of patients with ulcerative colitis and Crohn's disease compared with controls (16, 21, 22). Antigens derived from Escherichia coli 014 and another serotype, Escherichia coli 0119.K69 (B14) were also found to induce lymphocytes to become cytotoxic to colonic mucosa (22, 23). These findings led to the suggestion that these specific Escherichia coli serotypes are casually involved in the pathogenesis of inflammatory bowel disease.

In order to test this hypothesis, and because the intestinal Escherichia coli flora can contain a variety of serotypes over a period of time (24-26), it
was considered that an assay of antibodies to all currently accepted 159
Escherichia coli O-antigens in the serum of patients with ulcerative colitis
and Crohn's disease might give a better understanding of the bacterial role in
these patients, and also determine if the antigenic stimulus is limited to the
specific serotypes as previously reported, or if it is a widespread reaction.

(i) Escherichia coli antibodies in patients with IBD. - Sera from 30 pa-
tients with inflammatory bowel disease (IBD) (16 with Crohn's disease (CD) and
14 with ulcerative colitis (UC)) were assayed for the presence of antibodies
against 159 Escherichia coli O-antigens and compared with sera from 16 matched
control subjects (Table 4). The majority of patients with IBD had agglutinating
antibodies to a higher number of Escherichia coli O-antigens and in higher
titres than the control group. The number of positive agglutinins was 0-33 mean
13.8 in CD, 0-26 mean 7.9 for UC, and 0-7 mean 1.5 in controls. Eight patients
with IBD and arthropathy had antibodies to fewer O-antigens (0-7 mean 3.2). The
antibodies were in the IgG and IgM, in titres corresponding to original values.
No specific O-serotypes were associated with IBD. Common serotypes, R-plasmid
carrying serotypes, and those associated with shigella-like adult diarrhoea
were detected. 014 was detected only in five patients and 0119 in none. There
was no correlation between the number of Escherichia coli agglutinins and the
site and severity of the disease or type of therapy. It is suggested that the
presence of the high numbers of Escherichia coli antibodies is secondary to the
disease process and is unlikely to be causally involved in the pathogenesis of
the disease, but may play a role in the perpetuation of the disease and in the
extraintestinal complications (27).

Table 4. - Distribution of agglutinin titres against Escherichia coli in the
different patient groups

<table>
<thead>
<tr>
<th>N. of patients studied</th>
<th>Control 16</th>
<th>Crohn's disease 16</th>
<th>Ulcerative colitis 15</th>
<th>IBD + arthropathy 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agglutinin titres</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/200</td>
<td>14(11)</td>
<td>89(11)</td>
<td>52(9)</td>
<td>16(4)</td>
</tr>
<tr>
<td>1/400</td>
<td>6(4)</td>
<td>77(12)</td>
<td>40(11)</td>
<td>5(4)</td>
</tr>
<tr>
<td>1/800</td>
<td>3(2)</td>
<td>48(9)</td>
<td>23(8)</td>
<td>2(2)</td>
</tr>
<tr>
<td>1/1600</td>
<td>2(1)</td>
<td>5(5)</td>
<td>3(3)</td>
<td>0</td>
</tr>
<tr>
<td>1/3200</td>
<td>0</td>
<td>1(1)</td>
<td>1(1)</td>
<td>0</td>
</tr>
<tr>
<td>1/6400</td>
<td>0</td>
<td>2(1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total number</td>
<td>25</td>
<td>222</td>
<td>119</td>
<td>23</td>
</tr>
</tbody>
</table>

IBD: Inflammatory bowel disease.
Figures in parentheses: Number of patients in whom the corresponding number of
positive titres were found.

(ii) Antibodies to Bacteroides antigens of patients with IBD. - Heat
extracted antigens from seven species of Bacteroides were used to detect
antibodies in sera from 87 normal persons (group I), 15 patients with ulcerative
colitis (group II), and 12 patients with Crohn's disease. No significant
difference in the percentage of positive reactions was noted in the various
groups except for antigens prepared from B. vulgatus which successfully distin-
guished the controls and the other population and provided a means to separate
the patients in group II with active disease from those in remission at a P
value of 0.01. All sera from patients with Crohn's disease reacted with B.vulgatus antigens. The antibody response was predominantly an IgM (28). These authors suggested that these data provide no proof that B.vulgatus has an aetiological role in ulcerative colitis or Crohn's disease but merely a response to the continuous exposure of the immune system to the intestinal flora caused by the disruption of the cellular integrity of the gut.

(iii) Serum agglutinins to intestinal bacteria in Crohn's disease. - Several workers have noticed an increased incidence of serum agglutinins to intestinal bacteria in patients with Crohn's disease, of these the anaerobic Eubacteria and Peptostreptococcus (29, 30). A further study by Howells et al. (31) confirms this finding. This study shows a lower incidence of serum antibodies in Indian patients with infective colitis compared with patients with Crohn's disease. Blaser et al. (32) reported raised titres of serum antibody to seven bacterial pathogens but not to Campylobacter jejuni (33) in patients with Crohn's disease compared with controls. A non-specific increase in uptake of bacterial antigen across damaged mucosa probably explains these findings.

The role of infective agents in Crohn's disease.

(i) Transmissible agent. - Recently an athymic 'nude' model was developed to study the cause of Crohn's disease (34). Mucosal filtrate from Crohn's tissue were injected into athymic 'nude' mice. This produced murine B-cell lymphomas and lymph node hyperplasia. Similar mice injected with tissue filtrate from control patients or patients with ulcerative colitis did not develop lymphomas. However tissue filtrate from sarcoidosis produced a similar lymphoma.

Zucherman et al. (35), demonstrated that Crohn's disease induced lymphoma could be transmitted through 10 generations of athymic nude mice. Sera from patients with Crohn's disease reacted against 10 generations of Crohn's disease induced lymphomas by immunofluorescent assay. The antibody involved was probably IgG. Attempted characterization of the immunoreactive antigens in tissue from patients with Crohn's disease was carried out using immunoprecipitation techniques (36). Three major proteins of $10 \times 10^3$, $12 \times 10^3$ and $11 \times 10^3$ daltons were detected in Crohn's tissue but not in control or ulcerative colitis tissue after incubation with sera from Crohn's patients. It has still to be established whether these proteins are related to the cause of Crohn's disease.

(ii) Pseudomonas maltiphilia. - Parent and Mitchell (37) reported the isolation of cell wall deficient Pseudomonas maltiphilia from intestinal Crohn's disease and suggested that this organism might have a causal effect, but Graham et al. (38) using DNA hybridisation showed a more generalized association between cell wall deficient P.maltiphilia and both Crohn's disease and ulcerative colitis.

(iii) Mycobacterium species. - The possibility that Crohn's disease may be a Mycobacterial disease is an attractive idea because of the granulomatous nature of the lesion. Early studies utilizing conventional culture techniques gave negative results but in 1972, Patterson and Allen (39) provided experimental evidence of chronic Mycobacterial enteritis in ruminants which could serve as a model of human disease. This ruminant ileitis was caused by Mycobacterium paratuberculosis. These observations were followed by the isolation of Mycobacterium kansasii from one patient with Crohn's disease out of 27 (40). These workers also described pleomorphic organisms visualized by electron microscopy in other patients with Crohn's disease and ulcerative colitis, but this work was not confirmed by immunofluorescent studies (41). However, further evidence for the presence of acid-fast material in the tissue and lymph nodes of Crohn's disease was later provided by Stanford (42) and White (43).

More recently Chiodini et al. (44, 45) were able to culture colonies of previously unclassified Mycobacterium after a period of 3.5 to 18 months from three patients with Crohn's disease. This organism may be a subspecies or
biovariant of M. paratuberculosis or it may be a new species. Oral inoculation of this isolate to goats produced granulomas in the terminal ileum of these animals. Furthermore Thayer et al. (46) demonstrated exaggerated serum IgG response to crude preparations of this Mycobacterium in patients with Crohn's disease, but serum antibody levels showed no relation to disease activity. Isolation of this organism from patients with Crohn's disease was also achieved by a second group of workers (47), and subsequent application of DNA hybridisation techniques to the study of this organism revealed mycobacterial genomes in the tissue of patients with Crohn's disease. A M. paratuberculosis-like organism may therefore have a causative role in Crohn's disease but further studies are required.

We have started to study the occurrence of antibodies to M. paratuberculosis by an ELISA method. The wells were coated with a commercial preparation of a protoplasmic antigen derived from M. paratuberculosis at a concentration of 0.1 mg/ml (Allied Laboratories Inc). Doubling dilutions of patients serum to 1/2048 were applied. Goat anti-human IgG-AP conjugate was applied and incubated for 1 hour at 37°C. An alkaline phosphatase substrate (Sigma Laboratories) was added and the reaction stopped with IN NaOH. The O.D. was read at 405 nm.

So far sera from 24 patients have been examined. Fourteen of these patients had Crohn's disease. Three out of the 14 patients gave a strongly positive reaction (O.D. 405 nm > 2). The results of the other 11 patients ranged from O.D. 405 nm 0.039 - 1.727 mean 0.507. The remaining 10 patients included 4 in whom no intestinal abnormalities could be detected, and 1 case each of irritable bowel syndrome, abdominal pain, duodenal ulcer, carcinoma of the cervix, ulcerative colitis, and post infective irritable bowel. In one of these, a patient in whom no abnormal findings were detected, there was a very high O.D. 405 nm reading (> 2). The results from the other 9 ranged from O.D. 405 nm 0.031 - 1.933, mean 0.818.

Experimental production of ulcerative colitis.

Carrageenan induced colitis in animals provides an animal model in which the colonic lesions simulate those of acute ulcerative colitis in man. This model was used to determine whether there are any changes in the intestinal flora during the evolution of the disease and the effect of antibiotic therapy. Coliform concentrations increased from $10^{2.7}$ to $10^{7.4}$ per g during initial stages of colonic ulceration. Pretreatment with anticoliform antibiotics had no effect on the disease process although reduced the coliform counts. Pretreatment with metronidazole prevented the development of colitis. Thus it was observed that anaerobic bacteria play a role in the initial events of experimental colitis (48).

In order to test this finding further conventional guinea pigs given a solution of 5% (wt/vol) degraded carrageenan as the sole source of oral fluids, developed ulcerations of their caeca and large intestines within 30 days. No such lesions were detected in germ free pigs treated in identical manner, suggesting that an intestinal flora was necessary for the development of the intestinal lesions (49). Caecal microflora from carrageenan treated animals were cultured and tested in pools of 10 each, consisting of 10 bacterial strains. Germ free animals were inoculated with each pool and the results showed that two of the pools caused ulcerations in the presence of carrageenan and furthermore that a specific strain of Bacteroides vulgatus isolated from one of the pools, when mono-associated into germ free animals caused ulceration regardless of whether carrageenan was administered. These results suggest a bacterial involvement in disease development (49).

In order to define the role of B. vulgatus in this system, Onderdonk and his colleagues (50) immunised conventional guinea pigs with B. vulgatus before carrageenan treatment. Immunised animals developed both circulating antibody and positive skin tests for the homologous antigen. The results showed that both immune and non-immune animals had ulceration of caeca and colon but the severity of the ulceration was greater in the immune group which were fed live.
strains of *B. vulgatus* thus suggesting that immunisation with *B. vulgatus* enhances the severity of the carrageenan-induced colitis. The use of *B. fragilis* for immunisation and feeding did not produce any intestinal lesions, suggesting that there is some specificity for *B. vulgatus* in this model system.

In an effort to determine whether the effect of *B. vulgatus* was specific for guinea pigs, mice were immunized with *B. vulgatus* and associated with the organism. These mice developed lymphocytic infiltration of the lamina propria consistent with chronic inflammation. Similar results were not obtained with non-immune or *B. fragilis* associated mice. Thus antigenic stimulation of the gut may be one factor in the development of experimental ulcerated colitis. It was further demonstrated that certain strains of *B. vulgatus* are capable of provoking the more severe immunisation induced ulcerative colitis and that adoptive transfer of spleen cells from animals immunized with *B. vulgatus* to non-immune recipient animals is effective in transferring the immune enhancement demonstrated in activity immunized animals. These findings indicate that *B. vulgatus* strain specific factors are important to immune enhancement of experimental disease and also suggest an involvement of the cell mediated immune system in this animal model.

What is not known, however, is whether the same strain of *B. vulgatus* isolated from the experimental animal model is also associated with human disease.

Serum antibodies to *B. vulgatus*. - Antibodies to Onderdonk's strain of *B. vulgatus* were measured by an ELISA. *B. vulgatus* was grown for 48 hours at 37°C in an anaerobic chamber. After centrifugation and washing, different dilutions of the preparation were tested against the serum of a rabbit which had been immunized with Onderdonk's strain of *B. vulgatus*. Ultimately a 1/32 dilution was selected for coating the wells. Patient's serum was diluted 1/50 initially, and series of doubling dilutions made. A goat anti-human IgG-AP conjugate was added to the wells and the plates incubated for 60 min at 37°C. After addition of an alkaline phosphatase substrate, O.D. was read at 405 nm.

Sera were obtained from 21 patients with Crohn's disease and 8 patients with ulcerative colitis. The controls were 22 patients with other gastroenterological disease or without abnormality. Of the 21 patients with Crohn's disease, 5 gave a positive result (O.D. 405 nm > 1.0). A positive result was also given by 3 out of the 22 control patients. None of the 8 patients with ulcerative colitis were positive.

REFERENCES


