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Microbiologic Assessment of Tissue Biopsy Samples from Ileal Pouch Patients

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Tissue biopsy samples from patients with and without ileal pouches were examined by electron microscopic and microbiologic culture techniques to determine the numbers and types of microorganisms closely associated with or within the tissue biopsy samples. The disease status of each patient was determined by endoscopic and histopathologic methods. Of the 78 biopsy samples included in this study, 64 (82%) yielded obligately anaerobic and/or facultative bacteria when they were cultured. Fourteen of the 78 samples (17.9%) were negative by culture. Of the positive samples, 54 contained facultatively anaerobic bacterial species and 50 yielded obligately anaerobic species. The total counts for facultatively anaerobic bacteria for samples from patients with pouchitis were significantly greater than for samples from patients in control groups. In addition, the number of samples from patients with normal pouches that did not contain obligate anaerobes was significantly less than that from patients with pouchitis; 4 of 23 and 6 of 12 samples, respectively ($P < 0.043$). For samples in which organisms were detected, there was agreement with electron microscopic detection of bacteria in 23 of 27 samples, for an overall sensitivity of electron microscopy compared with that of culture of 85%. The qualitative studies resulted in the characterization of 273 isolates comprising 77 different phenotypes. The specificity of these findings in patients with ileal pouchitis is discussed.

Many inflammatory conditions of the gastrointestinal tract are grouped under the term inflammatory bowel disease. Much effort has been directed toward finding an etiology for these conditions. An inflammatory condition associated with the surgical construction of ileal reservoirs for patients with ulcerative colitis (and occasionally Crohn's disease or familial polyposis) has been described. This clinical syndrome, commonly known as pouchitis, has been identified in approximately 20% of patients with either a Kock continent ileostomy or a pelvic ileal pouch (4, 9). The symptoms of pouch inflammation, described in detail elsewhere (11, 18), include diarrhea, bloody discharge, fever, and characteristic histopathologic changes consistent with an acute inflammatory process. Clinical studies suggest that an increased number of obligate anaerobes (OAs) may be involved in the disease process, because of the favorable response of many patients to antimicrobial therapy directed against this group of microorganisms (6). Studies of the intestinal microflora of patients with Kock pouches, pelvic pouches, or conventional ileostomies have, however, yielded conflicting results. In one study of the microflora obtained from the reservoirs of patients with ileal pouch inflammation, the authors conclude that the total facultative anaerobe (FA) population is greater than that in ileal pouch patients without inflammation (8), while a second study reports that the FA population is greater (12). Additional studies indicated that in patients with ileoanal anastomosis there was a significant correlation between chronic inflammation and the number of FAs and between chronic inflammation and the number of OAs and total bacterial counts (17).

The present study was prompted by preliminary observations of tissue biopsy samples from pouch wall biopsy samples obtained from patients with ileal pouch inflammation. Transmission electron microscopic (TEM) examination of these specimens revealed bacteria within the tissues in some cases. We therefore undertook the examination of tissue biopsy samples from patients with and without ileal pouches by electron microscopic and microbiologic culture techniques to determine the numbers and types of microorganisms associated with or within the biopsy samples and to determine whether there were bacteriologic differences between patients with active pouch disease and various other patient groups.

MATERIALS AND METHODS

Patient groups. Patients from the Toronto General Hospital, Toronto, Ontario, Canada, were placed into one of five groups on the basis of endoscopic inspection, histopathologic evaluation by light microscopy of fixed tissue, and clinical findings. The methods of evaluating disease status, baseline information regarding current medications and the presence of symptoms suggestive of inflammation, and the correlation between endoscopic and histopathologic methods are described in detail elsewhere (11). Patients with either Kock or pelvic pouches were assigned to one of three groups, as follows. Pouch-normal patients (group I) were those patients with no evidence of pouch inflammation by either endoscopy or histopathology. Pouchitis patients (group II) were those patients in whom endoscopy showed evidence of pouch inflammation and histopathologic evaluation revealed moderate to severe acute inflammatory changes. Pouch-indeterminate patients (group III) were those patients in whom endoscopy showed evidence of pouch inflammation but histopathologic evaluation revealed

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mild or no acute inflammatory changes, or in whom endoscopy did not show evidence of pouch inflammation but histopathologic evaluation revealed moderate to severe acute inflammation.

Two control groups consisted of patients undergoing bowel surgery for unrelated reasons (group IV) and patients with conventional ileostomies and minimal evidence of inflammation (group V). A total of 78 patients were included in this study, with 23 in group I, 12 in group II, 14 in group III, 9 in group IV, and 20 in group V.

Biopsy samples. The pouch or ileostomy of all patients with pouches or conventional ileostomies was examined endoscopically by one of the investigators (R.S.M. or Z.C.) by using either a flexible or a rigid sigmoidoscope. All endoscopic findings were recorded, and the site of inflammation and its distribution were noted (focal or generalized). Six biopsy samples were obtained from each subject by using a flexible endoscopic biopsy forcep. Biopsy samples were obtained from the ileum below the fascial level in patients with conventional ileostomies. In patients with pouches, biopsy samples were obtained from the pouch, avoiding suture or staple lines. Two samples from each subject were submitted for light microscopic examination, two were processed for electron microscopy, and two were used for microbiologic studies.

To obtain biopsy samples of normal ileum, six specimens were obtained from the terminal ileum near the resection margin of patients undergoing right hemicolectomy. The specimen was examined by the surgeon to ensure that the small bowel was normal. Biopsy samples were taken in a manner similar to those described above.

Preliminary studies were performed to determine whether biopsy samples that had been frozen in liquid nitrogen before processing yielded total counts significantly different from those of samples received in the unfrozen state. It was determined that the average total counts were not significantly different (13). Therefore, samples were received frozen from the Toronto site for processing in Boston.

Microbiologic methods. Biopsy samples were placed in an anaerobic chamber (Coy Manufacturing, Ann Arbor, Mich.) prior to thawing. Each biopsy sample was weighed within a preweighed plastic tube and then subjected to six sequential washes in 1 ml of sterile phosphate-buffered saline (PBS; pH 7.2) which was vortexed gently for approximately 30 s. The biopsy sample was then homogenized in a sterile disposable hand-held homogenizer in 1 ml of PBS until no intact tissue was detected. Serial decimal dilutions of each sample were then made with sterile PBS. A 0.1-ml aliquot from the undiluted sample and the various dilutions were plated onto a variety of media to yield final dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. This technique yielded a sensitivity of between 500 and 1,000 organisms per g of tissue, depending on the initial weight of the sample. Brucella base blood agar (BMB), BMB containing 150 μg of neomycin per ml, and laked blood agar containing 10 μg of vancomycin per ml and 100 μg of kanamycin per ml were used for the recovery of OAs. Trypticase soy blood agar, chocolate agar, and MacConkey agar were used for the recovery of facultative species. Additional qualitative cultures of the undiluted homogenate were performed for the recovery of *Mycoplasma* spp. by using standard mycoplasma agar, for the recovery of *Campylobacter* spp. with Campy-thiol broth, and for the recovery of *Mycobacterium* spp. with Lowenstein-Jensen agar slants. Media were incubated in the appropriate gaseous environment for various lengths of time, as recommended for the recovery of the specific groups of microorganisms (7). Following incubation, quantitative plates were examined, colony types were enumerated, and subcultures were prepared for identification. An additional specimen obtained from the final wash solution for each biopsy sample was also plated for both OAs and FAs. Growth on these plates precluded any additional analysis of a biopsy sample. Identification of isolates was accomplished by previously described techniques (14). All colony counts were expressed as $\log_{10}$ CFU per gram of sample.

Electron microscopy. Biopsy samples were fixed for 2 h at room temperature in a mixture containing 2% paraformaldehyde, 2.5% glutaraldehyde, and 25 mg $CaCl_2$ in 0.1 M sodium cacodylate buffer (pH 7.4); washed overnight in 0.1 M sodium cacodylate buffer (pH 7.4); and postfixed in 1.5% collidine-buffered osmium tetroxide. Preceding dehydration in alcohol, the specimens were stained en bloc with uranyl acetate (2). Tissues were embedded in Epon 812. Blocks were cut with a diamond knife on an LKB IV ultratome, stained with lead citrate, and examined blindly by TEM by one electron microscopist (A.M.D.). All specimens were examined for the presence of intramural bacteria. Findings were recorded as the presence or absence of organisms, along with the morphologic characteristics of the organisms observed.

Comparison of electron microscopic and microbiologic findings. A comparison of the electron microscopic and microbiologic findings was made at the completion of the study, with an arbitrary cutoff of 10^6 CFU/g of tissue as the sensitivity level for a positive electron microscopic finding. This cutoff level was necessary because of the greater sensitivity of culture results than the actual observation of organisms in thin sections examined by TEM. If bacteria were cultured at this level or higher and organisms were seen by TEM, then a positive correlation was recorded. If organisms were cultured at less than 10^6 CFU/g of tissue and no bacteria were seen by TEM, a positive correlation was recorded. Finally, if bacteria were detected by culture in numbers less than 10^6 CFU/g and morphologically consistent organisms were seen by TEM, a positive correlation was recorded, since by chance alone it would be possible to detect organisms by TEM in some samples if the sections were obtained from an area where organisms were present.

Statistical analysis. Statistical evaluations of the results were performed with commercial statistics software (STATISTIX; NH Analytic Software, St. Paul, Minn.). Quantitative counts for bacterial populations were compared by use of Student's *t* test. Frequency observations were compared by chi-square analysis.

RESULTS

Microbiologic cultures. Of the 78 biopsy samples included in this study, 64 (82%) yielded obligately anaerobic and/or facultative bacteria when they were cultured. Fourteen of the 78 samples (17.9%) were negative by culture. Of the positive samples, 54 contained FA and 50 yielded OA species. A comparison by group of the number of cultures that did not contain any bacteria is shown in Table 1. It is of interest that the percentage of samples from group I (normal pouch) that did not contain OAs was significantly less than that of group II (pouchitis): 4 of 23 and 6 of 12 samples, respectively ($P < 0.043$). No significant differences were noted between group II and the other groups with regard to the number of cultures that were negative for OA. In contrast, none of the group II samples were culture negative for FA species, a finding that was significant ($P < 0.05$) for

TABLE 1. Comparison of negative cultures by group

Group	No./total no. tested (%)		
	OA negative	FA negative	Organisms negative
I (normal pouch)	4/23 (17) ($P < 0.42$)[a]	6/23 (26) ($P < 0.06$)	1/23 (4)
II (pouchitis)	6/12 (50)	0/12	0/12
III (indeterminate)	5/14 (36)	5/14 (36) ($P < 0.07$)	4/14 (29) ($P < 0.10$)
IV (normal)	5/9 (56)	5/9 (56) ($P < 0.006$)	3/9 (33) ($P < 0.063$)
V (ileostomy)	9/20 (47)	8/20 (40) ($P < 0.03$)	6/20 (30) ($P < 0.061$)

[a] P values are versus group II and were determined by chi-square analysis.

all but groups I and III ($P < 0.06$ and $P < 0.07$ versus group II, respectively). An examination of the samples that were negative for any organisms by culture revealed that both the control groups, groups IV and V, had a higher percentage of completely negative cultures than did group II ($P < 0.063$ and $P < 0.061$, respectively).

Comparison of bacterial culture and electron microscopy. A comparison of the electron microscopic findings of bacteria within the biopsy tissue and positive culture results is shown in Table 2. For samples in which organisms were seen by electron microscopy and which yielded a positive culture, 23 of 27 correlated, for an overall sensitivity of electron microscopy compared with that of culture of 85%. For groups I, II, and IV, there was 100% agreement between the two techniques, while the agreement for group III (pouch indeterminate) was 62.5%, and for group V (ileostomy) it was 50%. For biopsy samples that were negative by electron microscopy, 39 of 51 samples showed agreement between electron microscopic findings and culture results, a finding indicating that the specificity of electron microscopy versus that of culture was only 76%. It is noteworthy that for the pouch-indeterminate group, there was a correlation with culture of only 50% for the negative samples, compared with 71 to 100% for the other groups. An examination of the electron microscopic findings by group (Table 3) revealed that a similar percentage of samples from patients in groups I, II, and III had findings that were positive or negative by electron microscopy. However, both of the control groups (groups IV and V) yielded a lower number of positive observations and a corresponding higher number of negative findings. For the ileostomy group (group V) the findings were significantly different from those for group II (pouchitis) or the groups with pouches (groups I, II, and III) as a whole.

Comparison of total FA and OA counts. A comparison of the total OA and total FA counts for the various groups is shown in Fig. 1. The mean anaerobic counts ranged from 2.52 to 4.18 $\log_{10}$ CFU/g of tissue for the five groups. The total OA counts for the biopsy samples from patients with pouches were slightly higher than those for samples from the group with normal ileal tissue or for subjects with ileostomies; however, none of the differences approached statistical significance. In contrast, the total FA counts ranged from a mean of 1.63 $\log_{10}$ CFU/g of tissue for normal ileal tissue to 6.29 $\log_{10}$ CFU/g for biopsy specimens from patients with pouchitis. The total FA counts for biopsy samples from patients with pouchitis were significantly higher than those for the two control groups ($P < 0.03$, Student's t test) but not for the normal or indeterminate pouch groups (groups I and III, respectively).

Qualitative culture results. A total of 273 separate isolates were characterized from the 78 biopsy samples (mean of 3.5 isolates per biopsy sample). A total of 77 phenotypes were identified, which allowed 262 of the 273 isolates (96%) to be placed into 1 of 72 species and/or genus designations, while 11 (4%) of the isolates representing five phenotypes could not be placed into a genus or species by the methods used in this study. A comparison of the most commonly isolated organisms by group (Table 4) indicates that certain organisms were more frequently isolated from the pouch biopsy samples (groups I, II, and III) than from the control group biopsy samples (groups IV and V). All of the *Bacteroides vulgatus* isolates (frequency, 10 of 49 samples) came from the pouch biopsy samples, with no isolates of this organism coming either from normal ileal tissue or from the ileostomy biopsy samples (0 of 29; $P < 0.024$). If just the samples from the normal pouch group (group I) are compared with samples from the control groups, an even greater difference is noted ($P < 0.007$). Similarly, most of the *Escherichia coli* isolates were from the pouch biopsy samples (12 of 49), with only two isolates from the 28 control biopsy samples ($P < 0.01$). The isolation of *Bacillus* spp. from pouch samples was marginally significant, with 7 of 49 biopsy samples in the pouch groups and none of the control biopsy samples yielding this organism ($P < 0.085$).

Although the frequency of isolation of *Streptococcus* spp. of various types was not significantly different among the five groups, it can be seen that the highest counts for

TABLE 2. Comparison of electron microscopy and culture results

Group	No. culture positive/no. positive by electron microscopy (%)	No. in which culture had less than 10^6 CFU per g/no. negative by electron microscopy (%)
I (normal pouch)	10/10 (100)	10/13 (77)
II (pouchitis)	5/5 (100)	5/7 (71)
III (indeterminate)	5/8 (62)	3/6 (50)
IV (normal)	2/2 (100)	7/7 (100)
V (ileostomy)	1/2 (50)	14/18 (78)
All	23/27 (85)	39/51 (76)

TABLE 3. Frequency of observation of organisms seen by electron microscopy

Group	No. positive by electron microscopy/total no. (%)	No. negative by electron microscopy/total no. (%)
I (normal pouch)	10/23 (43)	13/23 (57)
II (pouchitis)	5/12 (42)	7/12 (58)
III (indeterminate)	8/14 (57)	6/14 (43)
IV (normal)	2/9 (22)	7/9 (78)
V (ileostomy)	2/20 (10)[a]	18/20 90)[a]

[a] $P < 0.035$ versus group II and $P < 0.009$ versus groups I, II, and III.

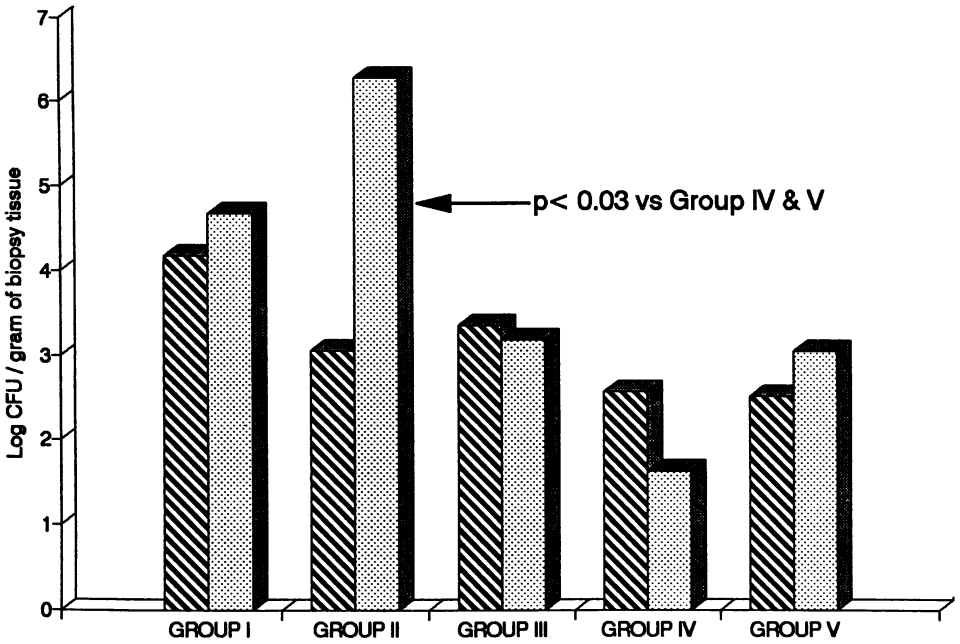

FIG. 1. Comparison of total bacterial counts from tissue biopsy samples. Groups: I, normal pouch; II, pouchitis; III, indeterminate; IV, normal; and V, ileostomy. ▨, obligate anaerobes; □, facultative species.

Streptococcus spp. were from the pouchitis group (group II). This finding is consistent with the higher counts of FA organisms from these biopsy samples in general compared with those from the control groups.

A complete listing of the various species isolated, the frequency of isolation, and the average counts is given in Table 5.

DISCUSSION

On the basis of the preliminary observations of bacteria within biopsy samples from patients with ileal pouchitis, we sought to determine whether viable organisms could be isolated from such samples. In addition, these studies were designed to measure differences in the types and numbers of closely associated organisms in biopsy material from patients with ileal pouch disease and from those without any manifestations of this disease. It is notable that the microbiologic studies could not differentiate between closely associated bacteria, as defined by the washing procedures, and organisms that were actually within the tissue. Thus, the occasional isolation of a variety of species is not surprising, given the complexity of the intestinal microflora. Our analysis of the data involves determination of whether quantitative or qualitative trends in the various sample groups are of interest for additional study.

One observation that can be made on the basis of these data is that patients with pouchitis all had some bacteria present in the biopsy samples, all had FAs (Table 2), and half had OAs. Isolation of facultative bacteria in every sample

TABLE 4. Frequency and mean count of bacteria for study group

Organism	% of samples (mean $\log_{10}$ CFU/g of sample) for group[a]:				
	I	II	III	IV	V
Staphylococcus spp.	54 (5.2)	75 (4.9)	54 (4.9)	33 (3.5)	53 (4.6)
Group D streptococci	21 (4.3)	25 (5.2)	8 (4.9)		21 (4.4)
Non-group A, B, D streptococci	8 (6.3)	17 (7.6)	23 (5.5)		16 (4.4)
Other streptococci	13 (3.6)			22 (3.7)	
All streptococci	46 (4.5)	42 (6.1)	23 (5.4)	22 (3.7)	37 (4.4)
Corynebacterium spp.	25 (5.2)	25 (7.6)	8 (6.0)	11 (4.7)	21 (6.0)
Bacillus spp.	21 (5.9)	17 (8.3)			
Actinomyces and *Arachnia* spp.	8 (4.7)	17 (7.5)	15 (5.1)	11 (5.2)	15 (4.4)
Bifidobacterium, *Eubacterium*, and *Lactobacillus* spp.	21 (4.7)	17 (6.4)	38 (4.2)	33 (5.2)	25 (4.4)
Propionibacterium spp.	8 (4.4)		8 (3.3)	11 (7.2)	10 (5.2)
Escherichia coli	25 (4.6)	25 (4.2)	31 (4.1)	11 (3.5)	5 (4.5)
Facultative gram-negative rods	13 (4.4)	25 (5.3)			11 (4.9)
Bacteroides spp.	48 (5.4)	25 (5.0)	29 (5.8)	22 (7.1)	21 (4.9)
Bacteroides vulgatus	29 (4.7)	17 (5.4)	8 (4.5)		
Fusobacterium spp.	4 (5.3)				

[a] Groups: I, normal pouch; II, pouchitis; III, indeterminate; IV, normal; V, ileostomy.

TABLE 5. Frequency distribution by genus and species

Organism	Mean count[a]	No. of observations[b]	Organism	Mean count	No. of observations
Actinomyces israelii	7.44	2	*Eubacterium moniliforme*	4.67	1
Actinomyces meyeri	4.42	2	*Eubacterium rectale*	4.22	1
Actinomyces odontolyticus	5.80	1	*Eubacterium saburreum*	7.70	1
Actinomyces viscosus	3.61	1	*Fusobacterium prausnitzii*	5.30	1
Bacillus spp.	6.56	7	*Gaffkya anaerobia*	4.22	1
Bacteroides spp.	4.09	5	*Haemophilus parahaemolyticus*	5.21	1
Bacteroides bivius	5.70	1	*Klebsiella pneumoniae*	4.65	3
Bacteroides capillosus	5.27	2	*Lactobacillus* spp.	5.34	4
Bacteroides coagulans	5.84	1	*Lactobacillus acidophilus*	5.11	1
Bacteroides distasonis	5.00	5	*Lactobacillus brevis*	4.41	2
Bacteroides fragilis	5.48	7	*Lactobacillus delbrueckii*	2.30	1
Bacteroides furcosus	3.77	1	*Lactobacillus fermentum*	5.41	1
Bacteroides multiacidus	6.52	1	*Lactobacillus minutus*	5.76	3
Bacteroides nodosus	5.70	1	*Lactobacillus plantarum*	4.96	3
Bacteroides oralis	4.05	1	*Peptostreptococcus prevotii*	4.04	1
Bacteroides ovatus	4.80	2	*Peptostreptococcus asaccharolyticus*	4.60	1
Bacteroides pneumosintes	6.04	2	*Peptostreptococcus productus*	5.55	1
Bacteroides ruminicola	5.61	4	*Peptostreptococcus saccharolyticus*	5.00	1
Bacteroides splanchnicus	3.96	1	*Propionibacterium acnes*	4.38	3
Bacteroides thetaiotaomicron	6.28	3	*Propionibacterium avidum*	5.03	1
Bacteroides uniformis	7.86	1	*Propionibacterium freudenreichii*	7.16	1
Bacteroides vulgatus	4.82	10	*Propionibacterium jensenii*	3.80	1
Bifidobacterium sp.	5.16	1	*Pseudomonas maltophilia*	4.99	3
Bifidobacterium bifidum	4.94	3	*Pseudomonas stutzeri*	6.40	1
Bifidobacterium breve	4.93	4	*Staphylococcus* spp.	4.74	42
Bifidobacterium infantis	4.57	3	*Staphylococcus aureus*	8.65	1
Bifidobacterium longum	3.77	4	*Streptococcus* spp.	3.63	3
Bifidobacterium magnum	4.81	2	*Streptococcus constellatum*	4.52	1
Candida albicans	4.43	1	Group B streptococcus	5.62	1
Clostridium bifermentans	4.22	1	Group D streptococci	4.59	13
Clostridium perfringens	5.22	2	Non-group A, B, D streptococci	5.76	10
Clostridium malenominitum	4.65	1	*Streptococcus intermedius*	4.02	4
Clostridium sartagoformum	5.31	1	Yeasts	2.93	1
Corynebacterium spp.	5.91	15	*Veillonella parvula*	4.82	4
Escherichia coli	4.21	15	Facultative gram-positive rod	4.22	1
Eubacterium sp.	4.55	1	Facultative gram-negative rod	4.47	6
Eubacterium aerofaciens	4.56	1	Anaerobic gram-positive cocci	3.53	1
Eubacterium lentum	4.83	3	Anaerobic gram-negative rod	4.28	2

[a] Mean count is expressed as $\log_{10}$ CFU per gram of tissue.
[b] There were a total of 78 observations.

from this group is statistically significant when compared with samples from all other groups except group III (indeterminate group). Although antimicrobial agents were used by some of the subjects in this study, the distributions of these subjects and the antibiotics used were similar for all groups and did not correlate with the presence or absence of bacteria in tissue biopsy samples. Despite the inability of the culture technique to define the relationship of the organism to the tissue itself, the correlation between the quantitative bacteriologic findings and the electron microscopic studies suggests a clear relationship. For biopsy samples that were positive by electron microscopic observation, the correlation with culture results was 100% for all but the indeterminate group. This finding may reflect the transitional nature of the indeterminate group, as judged microbiologically and by endoscopy and histopathologic evaluation. A similar, although not as dramatic, trend was noted for electron microscopy-negative samples. It is also important that the likelihood of seeing organisms by electron microscopy was higher if the sample was obtained from a patient with an ileal pouch, irrespective of pouch status.

Quantitative studies of OAs within ileostomy effluent generally have shown less than 10^7 CFU/g of sample (3). Studies of patients with ileal pouch disease reveal counts somewhat higher than this, ranging up to 10^8 CFU/g of sample in patients with active pouchitis. However, in contrast to the normal ratio of OAs to FAs of 100:1 to 1,000:1 within the large intestine, FAs outnumber OAs by a considerable amount in both noninflamed and inflamed pouches, often reaching levels of 10^9 CFU/g of sample (8).

In the present study, the most dramatic findings were total FA counts in biopsy samples from patients with pouchitis, which were, on average, 1,000 to 10,000 times higher than those noted in the control groups and 50 to 500 times higher than those noted in the other patients with ileal pouches. The total OA counts were slightly higher in the pouch-normal group than in the other patient groups but were otherwise unremarkable. These findings are at variance with bacteriologic studies of lumen contents that indicate that total OA counts are higher in patients with pouchitis than they are in controls (8). If the numbers and types of bacteria present in tissue samples merely reflect the relative types and numbers of organisms present in lumen contents, then we should have observed considerably higher OA counts in samples from patients with pouchitis because the luminal OA populations are reportedly higher in these patients than they are in those

without pouchitis. In fact, just the opposite trend was observed, suggesting that the high FA population in tissue biopsy samples is more than a passive reflection of bacterial concentrations in the lumen.

If we assume that both the previous bacteriologic studies of the lumen and the present tissue biopsy studies are accurate, then the data suggest that more than a passive process is occurring microbiologically. Indeed, the isolation rates for various species, particularly gram-positive organisms within the biopsy samples, suggests there is specificity to the findings in patients with ileal pouches, even though a single etiologic culprit has not been identified. *B. vulgatus* has been implicated in experimental inflammatory bowel disease (1, 15), while *E. coli*, *Streptococcus* spp., and *Bacillus* spp. have also been identified as being tissue-invasive organisms under the right conditions (10). It has also been suggested that the peptidoglycan of gram-positive organisms may cause tissue irritation and inflammation. On the basis of the available data, it appears that the tissue microbiologic findings do not represent passive translocation of luminal organisms into tissues that have been damaged by an inflammatory process. Although the actual mechanism by which inflammation occurs has not been identified, one would expect to find bacteria present in the same relative proportions noted in the lumen if passive translocation into the tissue were occurring.

Among the microbiologically mediated mechanisms that should be considered as etiologic possibilities for ileal pouch disease is the overproduction of specific short-chain fatty acids (SCFAs) that may lead to a pH considerably lower than that normally found in the large intestine, accompanied by growth of relatively acid-resistant organisms, including *Streptococcus* spp. This situation, equivalent to rumen overload in ruminant animals, can lead to serious inflammation of the rumen tissue, with invasion of the tissues by a variety of microbial species (19). Is it possible that the creation of a pouch mimics, to some extent, the stasis that can occur microbiologically within the rumen? Alternatively, one study of the intestinal contents of patients with diversion colitis suggests that the absence of the principal end products produced by OAs, SCFAs, are related to the development of inflammation and that restoration of the concentrations artificially is beneficial in treating these patients (5). Studies are in progress to measure the pH and SCFA concentrations in the lumina of patients with infected pouches to determine whether it is possible that some patients undergo a dysbiotic change similar to that of patients described here in which an imbalance of SCFAs may account for the inflammatory changes. This dysbiosis may, in fact, be due to either the absence or the overproduction of the normal levels of SCFAs, which are known to be inhibitory to facultative organisms such as coliforms. Such conditions may be caused by the mechanical alteration of the normal fecal stream or a variety of biologic factors.

An additional hypothesis that is being considered is the possibility that overproduction of bacterial enzymes, such as sulfatases, provides an appropriate substrate for H_2S producers, a product known to be quite toxic to tissues.

Clinical reports of the efficacy of antianaerobe drugs, particularly metronidazole, lend support to the hypothesis that OAs are involved in the overall mechanism of pouch inflammation. Metronidazole has also been shown to suppress the facultative gram-negative population as well as the anaerobic microflora both in vitro and in vivo (16).

Clearly, additional work is necessary to document the role of the various bacterial populations in the disease process. The studies that are under way in our laboratories may help to define the population(s) of interest for further examination.

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