Fecal Profiles of Anaerobic Microflora of Large Bowel Cancer Patients and Patients with Nonhereditary Large Bowel Polyps

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ABSTRACT

It has been postulated that the intestinal anaerobes play a role in the etiology of large bowel cancer. This study was designed to characterize and compare the fecal anaerobes of patients with large bowel cancer, patients with nonhereditary large bowel polyps, and healthy control subjects. Although some distributional variations of the anaerobic genera were observed among the study groups, significant differences in fecal anaerobic microflora and total aerobic counts were not noted. This suggests that taxonomic grouping of fecal bacteria is an inadequate measure of relative risk of developing large bowel cancer. However, the fecal microbial 7α-dehydroxylase and cholesterol dehydrogenase activities of large bowel cancer patients and patients with nonhereditary large bowel polyps were significantly higher than those of healthy control subjects. On the other hand, no significant difference in fecal microbial β-glucuronidase activity was noted among the study groups. It may be that assessment of the total metabolic activities of the intestinal microflora will provide a better understanding of their potential role in the genesis of large bowel cancer.

INTRODUCTION

The worldwide distribution of large bowel cancer incidence and mortality shows great variability, being much more common in North America and western Europe than in east Africa, India, and Japan (2, 10, 50). Migrants from low- to high-incidence areas soon assume the incidence rates indigenous to the area (21, 44, 48). This implies that environmental factors are more important than genetic factors. The most commonly suggested environmental factors associated with the development of large bowel cancer are dietary habits (20, 30, 38, 45). Various nutritional factors, including lack of fiber in the diet (7, 33) and high levels of refined carbohydrates (12), animal fat (48, 50), and beef (21), have been advanced as significant factors in the variance of large bowel cancer rates. The incidence of large bowel cancer among American Mormons and Seventh-Day Adventists is less than in the general population eating a mixed Western diet (14, 31, 49).

Diet may influence the fecal microflora (3, 4, 6, 8, 25, 40, 43, 47), fecal bile acids and neutral sterol profiles (23, 25, 27, 42), and fecal bacterial enzyme activities in man (32, 39). The intestinal microflora may metabolize bile acids to carcinogenic or/and cocarcinogenic compounds (1, 27). These secondary bile acids have been reported to act as colon tumor promoters but not complete carcinogens in an animal model (41). In one study, Hill et al. (28) reported significant differences in the fecal microbial flora of large bowel cancer patients. Since the putative carcinogenic or cocarcinogenic metabolite(s) of bile acids is formed by bacterial transformation, this study was designed to determine whether differences in fecal bacterial profiles existed among patients with large bowel cancer, patients with nonhereditary large bowel polyps, and control subjects.

MATERIALS AND METHODS

The study groups consisted of volunteers from the New York metropolitan area who were consuming a normal mixed Western diet. For the bacteriological studies, the control subjects consisted of 7 healthy adults (3 males and 4 females with a mean age of 46 years) without evidence of intestinal problems. In addition, 13 patients (8 males and 5 females with a mean age of 58 years) with diagnosed colorectal carcinoma and 7 patients (2 males and 5 females with a mean age of 38 years) with nonhereditary large bowel polyps were studied. None of the subjects had been on antibiotic treatment or other therapy for at least 4 weeks prior to collection of the fecal samples. No laxatives or other cathartics were used within 1 week of stool collection, and in all cases the specimens were obtained before bowel preparation was begun. None of the patients had visually observed blood contaminating the feces. Each individual gave informed consent.

Fresh fecal specimens were collected in sterile plastic bags at the hospital or at the residence of the subject by a nurse-coordinator and immediately placed in a disposable GasPak anaerobic system (Baltimore Biological Laboratory, Cockeysville, Md.). They were then transported to the laboratory, coded, and processed under strictly anaerobic conditions using a Virginia Polytechnic Institute anaerobic culture system within an hour of defecation. After thorough mixing of the specimen under a stream of oxygen-free gas (90% N₂,10% CO₂), approximately 1.0-g samples (wet weight) of the fecal homogenate were suspended in each of 2 preweighed 9.0-ml dilution blanks (Scott Laboratories, Fiskeville, R. I.) containing a few sterile glass beads. After each tube was reweighed, the contents were homogenized thoroughly on a vortex. A duplicate set of dilution series was prepared in prereduced, anaerobically sterilized dilution blanks to 10⁻⁸ dilution. One set was heated in a hot water bath at 80° for 10 min and then inoculated onto reinforced clostridial agar roll tubes and Trypticase soy blood agar plates. Each dilution series in duplicate was
inoculated anaerobically into brain-heart infusion agar and rumen fluid-glucose cellobiose agar roll tubes. Trypticase soy blood agar plates, chopped meat glucose, chopped meat, and thioglycollate fluid. The anaerobic brain-heart infusion agar, rumen fluid-glucose cellobiose agar, and reinforced clostridial agar roll tubes were used to quantitate total anaerobic and clostridial counts, respectively. Corrected colony counts based on dry weight were obtained by the methods described in the Virginia Polytechnic Institute Anaerobe Manual (29). Duplicate direct microscopic clump counts were prepared from the 10⁻³ dilution and compared to the observed colony counts to determine the percentage of recovery of anaerobes. Trypticase soy blood agar plates were also inoculated and incubated aerobically at 37° for 24 to 48 hr and used to quantitate the fecal aerobes based on colony counts.

The dilution series were kept on ice during the procedures and were then passed into a Coy anaerobic chamber (85% N₂:10% CO₂:5% H₂) containing plates of general and selected media anaerobically reduced for a minimum of 24 to 48 hr. The dilution series were inoculated in duplicate onto the following media: Trypticase soy blood agar, Forget-Frederette agar, Clostrisiel agar, phenylethyl alcohol blood agar, neomycin blood agar, kanamycin-vancomycin blood agar, neomycin-vancomycin blood agar, Columbia colistin nalidixic acid agar, Veillonella-vancomycin agar, sulfite polymyxin sulfadiazine agar, and brain-heart chloramphenicol cycloheximide agar. The plates were allowed to incubate at 37° in vented GasPak jars for 5 to 7 days, after which primary isolation of anaerobes was achieved by picking about 50 representative colonies in the anaerobic chamber to chopped meat and chopped meat glucose media. After good growth, generally 48 hr, the isolates were stained and, if pure, transferred to peptone yeast glucose and chopped meat agar slants. Definitive identification of each isolate was based on Gram reaction (Kopeboff modification), morphology, spore test, gas-liquid chromatography profiles of ether, and methylated extracts from peptone yeast glucose cultures, with the use of a Hewlett-Packard gas chromatograph fitted with a Resoflex column, and the necessary biochemical determinations (29), with both the API 20 anaerobic system (Analytab Products, Inc., Plainview, N. Y.) and commercially prepared anaerobic media (Scott Laboratories, Fiskeville, R. I.).

Fecal bacterial 7α-dehydroxyrase, cholesterol dehydrogenase, and β-glucuronidase activities in patients with large bowel cancer, patients with nonhereditary large bowel polyps, and healthy control subjects were determined as previously described (32). The volunteers analyzed for fecal bacteriological profiles are included in these assays.

RESULTS

The general characteristics of the feces obtained for each group of subjects are presented in Table 1. There was no difference in the percentage of moisture content of the feces and in the daily fecal dry matter excretion among the 3 groups. The mean total microscopic count of the fecal flora was slightly but not significantly higher in large bowel cancer patients and patients with nonhereditary large bowel polyps than in controls. Although 2 colorectal cancer patients had relatively low counts of total anaerobes, the mean total anaerobic count was highest in this group of patients. The low counts of fecal anaerobes in these 2 colorectal cancer patients may only reflect our inability to culture the isolates rather than the absolute concentrations of fecal anaerobes. The percentage recovery of fecal anaerobes compared to the microscopic count was a mean value of 64% or better. The mean concentration of fecal aerobes was highest in the controls, but individual variation and small sample size negate statistically significant differences among the groups. The ratio of anaerobes to aerobes was 4.7 and 8 times greater among patients with large bowel cancer and nonhereditary large bowel polyps compared to controls, respectively.

The total mean concentration of the fecal anaerobic genera recovered and the number of subjects in each group from whom these genera were isolated are presented in Table 2. Although some variation is evident, in general, the concentrations of fecal anaerobes among the groups were comparable.

The percentage compositions and distributions of the fecal anaerobic flora in the 3 study groups are presented in Table 3. Bacteroides, Bifidobacterium, Clostridium, Eubacterium and Lactobacillus species were most commonly isolated. Anaerobic cocci, Propionibacterium, and Fusobacterium species were found less commonly, and other genera were encountered infrequently. Despite great diligence, a number of specimens contained isolates that we were unable to characterize. These organisms are grouped together in Table 2 as “unidentified.” Species of Bifidobacterium, although generally constituting a predominant

### Table 1

<table>
<thead>
<tr>
<th>Fecal constituents of patients with large bowel cancer or nonhereditary large bowel polyps and healthy controls</th>
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<tr>
<td></td>
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<tr>
<td>% dry matter</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Control subjects (7)</td>
</tr>
<tr>
<td>Large bowel cancer patients (13)</td>
</tr>
<tr>
<td>Nonhereditary polyp patients (7)</td>
</tr>
</tbody>
</table>

⁰ Numbers in parentheses, number of subjects.

ⁱ Mean ± S.E.
group of organisms in control subjects and in patients with nonhereditary large bowel polyps, were less frequently isolated from large bowel cancer patients; species of Fusobacterium were more frequently isolated from healthy controls. Species of Bacteroides were found in highest concentrations in all but one specimen, and constituted the bulk of the anaerobic fecal flora in each group of subjects (40 to 52%). Bifidobacterium and Eubacterium were recovered in nonhereditary large bowel polyps, were less frequently isolated from large bowel cancer patients; species of Fusobacterium, Propionibacterium butyricum, Clostridium tertium, and Clostridium butyricum, Clostridium sartago for of Clostridium, i.e., Clostridium butyricum, Clostridium tertium, were greater in large bowel cancer patients. One or more of the lecithinase-negative species of Clostridium tertium was recovered from the stools of 5 of 13 large bowel cancer patients (38.5%), whereas only 1 of 7 control specimens (14.3%) and 1 of 7 stools of patients with nonhereditary large bowel polyps (14.3%) yielded similar organisms. Moreover, the mean concentration of this group of organisms was 2 logs greater in large bowel cancer patients than in the other groups. These differences, however, were not statistically significant.

Despite a thorough quantitative and qualitative study of the fecal anaerobic flora of control subjects and patients with large bowel cancer or nonhereditary large bowel polyps, no clearly discernible statistically significant relationships between the fecal profile of the bacterial flora and colon cancer were evident. However, the metabolic activities of the fecal bacterial flora among these groups of subjects differed significantly. Although the mean fecal bacterial 7α-dehydroxylase and cholesterol dehydrogenase activities were not significantly different between patients with large bowel cancer and nonhereditary large bowel polyps, their mean activities were significantly increased in both groups compared to healthy control subjects (Table 4). β-Glucuronidase activities, however, were similar among the study groups (Table 4). The data presented in this series represent an elaboration of results previously published (32) and include additional large bowel cancer patients.

**DISCUSSION**

The present study does not suggest any obvious shift in the profile of the microbial flora in individuals at increased risk for large bowel cancer. Finegold et al. (16) observed significantly different concentrations of some fecal organisms in colonic polyp patients and matched controls, but the organisms differed from those found to be different in a previous study of Japanese Americans or Japanese on...
Japanese) were compared, the former group was found to eating a traditional Japanese diet (15, 16, 35), no single organism or group of organisms was characteristic of high Western diets) and low-risk populations (rural Japanese, flora were observed between high-risk populations (polypium, among subjects from low-risk areas (36). Steroid types or numbers of bacteria in their stools and those of the latter group had more aerobic streptococci and entero bacteria (1, 27). Further studies by this group reveal more concentrations of the nuclear-dehydrogenating clostridia in patients with large bowel cancer.

However, work reported from England (1, 9, 11, 24, 27, 36) suggests differences in the fecal microflora of high- and low-risk populations. When high-risk subjects (British and American) and low-risk subjects (Ugandans, Indians, and Japanese) were compared, the elder group was found to have more fecal Bacteroides and Bifidobacterium, whereas the latter group had more aerobic streptococci and enterobacteria (1, 27). Further studies by this group reveal more Gram-negative anaerobes (Bacteroides and Fusobacterium) in subjects consuming a Western diet, and a greater proportion of Gram-positive organisms, especially Eubacterium, among subjects from low-risk areas (36). Steroid nuclear-dehydrogenating clostridia have been characterized in the stools of high-risk populations more frequently than in low-risk groups (24, 28).

Although some minor quantitative variations in intestinal flora were observed between high-risk populations (polyp patients and North Americans) and low-risk populations (rural Japanese, Africans of the Tswana, and Japanese-Americans eating a traditional Japanese diet (15, 16, 35)), no single organism or group of organisms was characteristic of high- or low-risk groups. Notwithstanding the unequivocal epidemiological clues of the importance of diet in large bowel cancer (2, 10, 21, 48, 50), attempts to alter the composition of the fecal flora by alterations in dietary intake have generally proved unsuccessful (13, 22, 35). Yet marked differences can be demonstrated among the flora of individuals (34-36).

The observed elevation in enzyme activity of the fecal flora to metabolize bile acids (7α-dehydroxylase) and cholesterol (cholesterol dehydrogenase) in patients with large bowel cancer or nonhereditary large bowel polyps compared to that in healthy control subjects may be due to altered physiological conditions in the colon affecting the bacteria that act on bile acids and cholesterol, or to quantitative and/or qualitative alterations in the cholesterol and bile acid-degrading anaerobic bacteria. Although the bacteriological profiles generated in this study do not appear to substantiate significant differences in the flora per se, this study and others (5, 9, 26, 28, 37, 46) have indirectly confirmed differences in fecal bacterial enzyme activities as indicated by the fecal excretion of bacterial degradation products of bile acids and cholesterol. In contrast, no differences in the fecal bacterial β-glucuronidase activity were observed among the study groups. Previous studies (19, 39) have demonstrated a relationship between diet and fecal microbial β-glucuronidase activity. In the present study, all volunteers, including the patients, were consuming a mixed Western diet, suggesting that the composition of the diet is a major factor in determining the bacterial β-glucuronidase activity.

An important point that arises from these studies is that taxonomic grouping of bacteria may be inadequate when measuring the effect of diet on the intestinal microflora. Since the intestinal bacteria contain many adaptive and constitutive enzymes, the metabolic activity of the gut bacteria can be modified appreciably by diet or other environmental factors. It appears that the overall metabolic activities of intestinal bacteria, regardless of species, need to be examined under different dietary conditions, as well as in groups consuming different diets, to understand the role of gut bacteria in the etiology of colon cancer. Such an

Western diets (15). A recent study by Finegold et al. (17) demonstrated that Seventh-Day Adventists had fewer Clostridium septicum and C. tertium isolates than did non-Adventists. Although the amount of fecal secondary bile acids and β-glucuronidase activity is decreased in vegetarians (42), no significant difference was found between the types or numbers of bacteria in their stools and those of the control group. Several studies (13, 15, 16, 18, 22, 34, 46), therefore, support our finding that gross differences in the composition of the fecal flora are not apparent between high- and low-risk populations. These data are not in complete agreement with those of Hill et al. (28), although, like Finegold et al. (15, 16), we report a slightly higher frequency of the lacticase-negative, nuclear-dehydrogenating clostridia among large bowel cancer patients than control subjects. Hill et al. (28) have found a correlation between bile acid metabolites and increased concentrations of the nuclear-dehydrogenating clostridia in patients with large bowel cancer.

### Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>7α-Dehydroxylase</th>
<th>Cholesterol dehydrogenase</th>
<th>β-Glucuronidase</th>
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<tbody>
<tr>
<td>Large bowel cancer patients</td>
<td>74.0 ± 6.2 1,2 (20)</td>
<td>59.8 ± 6.0 1 (15)</td>
<td>255 ± 40 1 (20)</td>
</tr>
<tr>
<td>Nonhereditary large bowel polyp patients</td>
<td>53.4 ± 6.2 1 (9)</td>
<td>32.3 ± 4.9 1 (6)</td>
<td>192 ± 48 1 (10)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>38.3 ± 2.9 2 (26)</td>
<td>20.8 ± 2.7 2 (15)</td>
<td>257 ± 35 2 (24)</td>
</tr>
</tbody>
</table>

1 7α-Dehydroxylase activity is expressed as percentage of cholic acid microbially converted to deoxycholic acid per 100 mg dry feces per 2-hr incubation; cholesterol dehydrogenase activity is expressed as percentage of cholesterol microbially converted to coprostanol and coprostanone per 100 mg dry feces per 24-hr incubation; β-glucuronidase activity is expressed as μg phenolphthalein glucuronide hydrolyzed per mg protein per 4-hr incubation.

2 Mean ± S.E.

3 Mean with a differing numbered superscript, 1 and 2, between groups in a vertical row is significant, p < 0.05 (Student's t test).

4 Numbers in parentheses, number of subjects analyzed.
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approach has been proposed by several investigators (13, 19, 32, 35). Elucidation of the metabolic activities of the organisms should provide a better understanding of the potential role of the intestinal microflora in the genesis of large bowel cancer. Correlations between the composition of the fecal microbial flora, fecal metabolites, and bacterial enzyme activities are being investigated.

ACKNOWLEDGMENTS

The authors wish to thank C. Sharma, B. Schimpf, and A. Molless for their expert laboratory technical assistance; C. Gustafson, R. N., nurse-coordinator for specimen collection; D. Conroy for manuscript preparation; and the volunteers as well as Dr. M. Stearns and Dr. P. Sherlock and staff of Memorial Hospital, New York, who collaborated and cooperated in this study.

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