Unusual *Sphaerophorus* Species from the Large Intestine of Man

TED A. PEARSON AND EDWARD BALISH

*Medical Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee 37830*

Received for publication 23 December 1969

An obligately anaerobic, gram-negative microorganism identified as a *Sphaerophorus* species was recovered from the fecal material of two cancer (chronic myelogenous leukemia and idiopathic thrombocytopenia) patients receiving cobalt radiation therapy. The organism, isolated on sheep blood-agar, exhibited extreme pleomorphism (rods, filaments, and spheroids) and was a major component of the anaerobic fecal microflora. In one patient the numbers of *Sphaerophorus* species (designated as isolate 6-13-68), *Bacteroides* species, and *Clostridium perfringens* declined after irradiation; however, they were stable in this same patient after a second therapeutic dose of radiation. The numbers of anaerobes in the other patient remained fairly consistent after radiation. The biochemical and morphological characteristics and carbohydrate fermentation reactions of isolate 6-13-68 most closely resembled those of *Sphaerophorus ridiculosis*.

The predominant components of the fecal microflora of normal humans are the anaerobic, nonsporeforming gram-negative bacilli of the family *Bacteroidaceae* (1, 2, 4, 9). This heterogeneous group is composed almost entirely of members of the genus *Bacteroides* and members of *Sphaerophorus* and *Fusobacterium* to a lesser degree. The gram-negative anaerobes may sometimes outnumber the coliforms by 10 to 1000. In this study, we determined the levels of *Sphaerophorus*, designated as isolate 6-13-68, and other anaerobic microorganisms in the feces of two patients before, and for several weeks after, cobalt radiation therapy.

**MATERIALS AND METHODS**

Culture methods for fecal material. A weighed sample of feces (0.5 to 1.5 g) was placed in 100 ml of sterile NaCl (1%, w/v) and agitated until uniformly suspended. This initial dilution of feces, used as a 1:100 dilution, was further diluted with sterile NaCl to 10^-4. A portion (0.1 ml) was spread on dry agar plates with a bent glass rod, immediately placed in anaerobe jars, and incubated for 3 to 7 days at 37°C.

**Bacteria.** *Sphaerophorus fredonii* (9817) and *S. varius* (8501) were obtained from the American Type Culture Collection. We isolated strain 6-13-68 from both patients. The isolates from both patients possessed identical morphological and biochemical properties. Cultures were maintained in thioglycollate broth (BBL) and transferred every 7 days.

**Anaerobiosis.** We used Gaspak anaerobe jars (BBL) with an atmosphere of 90% hydrogen and 10% carbon dioxide. Methylene blue indicators (BBL) were always used to insure removal of oxygen from the jars.

**Media for characterization.** Blood-agar plates with 10% defibrinated sheep blood (BBL) were used to demonstrate hemolysis. We studied carbohydrate fermentation in basal thioglycollate broth containing (grams per liter): Trypticase, 15; Phytone, 5; sodium thioglycollate, 0.5; cysteine, 0.125; agar, 0.70; and fermentable substrate, 10. To measure acid production, we compared the change in pH after 72 hr of incubation of thioglycollate broth cultures with and without the test substrate. Indole production and nitrate reduction were determined in Indole-Nitrite medium (BBL), hydrogen sulfide production in S I M medium (BBL), and gas and odor production in thioglycollate broth. For more reduced conditions, all liquid media contained sodium thioglycollate (BBL) at a concentration of 0.05% (w/v), and were boiled and cooled just before use.

To detect hemagglutinins in fresh isolates, we mixed equal amounts of microorganisms and sheep erythrocytes (2% in normal saline) on glass slides. The organisms were grown for 24 hr on sheep blood-agar plates, removed with sterile cotton swabs, and suspended in NaCl (1%, w/v) before testing.

**RESULTS**

We isolated 6-13-68 from patient A (Table 1) in concentrations of 10^9 to 10^10 viable cells per gram of feces (dry weight). Throughout the 4-week study, the numbers of isolate 6-13-68 and *Bacteroides* were fairly consistent; three samples (days -4, +4, and +10) of feces did not contain isolate 6-13-68 at a 10^-4 dilution (the lowest dilution plated). Isolate 6-13-68 and *Bacteroides*...
TABLE 1. Anaerobic fecal microflora of patient A treated with a single dose of 242 rads of splenic irradiation

| Time (days) | Bacteroides sp. | Isolate 6-13-68 | C. perfringens |
|-------------|-----------------|-----------------|---------------|
| −4          | <4<sup>a</sup>  | <4              | 6             |
| 0           | 10              | 9               | 7             |
| +4          | <4              | <4              | <4            |
| +6          | 10              | 10              | 9             |
| +10         | 10              | <4              | 5             |
| +12         | 7               | 6               | 6             |
| +14         | 10              | 10              | <4            |
| +23         | 9               | 9               | 4             |

<sup>a</sup> Log<sub>10</sub> of viable microorganisms per gram of stool (dry weight).

TABLE 2. Anaerobic fecal microflora of patient B before and after 150-r total-body irradiation<sup>a</sup>

| Time (days) | Bacteroides sp. | Isolate 6-13-68 | C. perfringens |
|-------------|-----------------|-----------------|---------------|
| −3          | 9<sup>b</sup>   | 9               | 7             |
| 0           | 8               | <4              | 8             |
| +3          | 8               | 8               | 9             |
| +8          | 9               | 9               | <4            |
| +10         | 9               | 5               | <4            |
| +18         | <4              | <4              | 4             |
| +24         | <4              | <4              | <4            |
| +31         | <4              | <4              | <4            |
| +45         | <4              | <4              | <4            |

<sup>a</sup> Patient was irradiated in June 1968 with 60Co at 1.5 r/hr in a special low dose rate facility.

<sup>b</sup> Log<sub>10</sub> of viable microorganisms per gram of stool (dry weight).

species were his predominant fecal bacteria. *Clostridium perfringens* was also present, but in lower numbers than the gram-negative anaerobes.

Patient B, with a predominance of isolate 6-13-68 in his fecal microflora, received therapeutic total-body irradiation (TBI) on two occasions. In June 1968, he was exposed to 150-r TBI (1.5 r/hr). The numbers of anaerobic microorganisms isolated before, during, and after the first total-body exposure of patient B are shown in Table 2. *Bacteroides* species and isolate 6-13-68 were consistently recovered before, and for about 2 weeks after, irradiation; however, on day 18, the numbers of *Bacteroides* and isolate 6-13-68 dropped below 10⁴ per gram and were not recovered for 4 weeks. *C. perfringens* was also present in high numbers and disappeared at the same time. At 10 months later, this patient was irradiated a second time with 150-r TBI at 1.5 r/hr, and 6-13-68 was again a predominant component of the anaerobic microflora at concentrations of 10⁴ to 10⁶ (Table 3). Isolate 6-13-68 and *Bacteroides* were isolated more consistently (only two samples were negative) during the second study than during the first. Although not isolated in consistently high numbers, *C. perfringens* was also present in the feces of patient B.

Colonies of isolate 6-13-68 on sheep blood-agar have a fried-egg shape (Fig. 1). They are circular, undulate, brownish-gray, possess a slight metallic gray sheen in the raised central portion of the colony, and are 3 to 6 mm in diameter after 96 hr of incubation at 37 C.

The morphology of isolate 6-13-68 is shown in Fig. 2. Short bacilli, long bent bacilli, long bacilli with blebs or swellings, and large spheroids were all present in the same thioglycollate broth.

FIG. 1. Colonial morphology of isolate 6-13-68 after 96 hr of anaerobic incubation on sheep blood-agar.
culture grown for 24 hr at 37 C. After the organism had been subcultured repeatedly, it grew as regular bacilli (3 to 6 μm long).

The physiological characteristics of isolate 6-13-68 were compared with those of S. varius (§ 8501) and S. freundii (§ 9817), which were obtained from the American Type Culture Collection (Table 4). The data for S. ridiculosus were taken from Prevot (7). All four are found in the intestinal tract of man, produce gas, foul odor, and hydrogen sulfide, and are extremely pleomorphic. Only S. varius produced indole and only S. freundii reduced nitrate to nitrite. Colonies of isolate 6-13-68 were beta hemolytic on sheep blood-agar only after they were exposed to the atmosphere for 72 hr at room temperature. Fresh isolates of 6-13-68 also possess a hemagglutinin for sheep erythrocytes.

The capacity of some Sphaerophorus species to ferment sugars, as determined by acid production from various carbohydrates, is shown in Table 5. The data for S. ridiculosus were taken from Prevot (7). Sphaerophorus varius fermented only glucose and fructose; isolate 6-13-68 and S. freundii produced acid from several carbohydrates (Table 5).

Isolate 6-13-68 did not produce acid from sucrose but S. freundii did; isolate 6-13-68 fermented maltose and S. freundii did not. Sphaerophorus ridiculosus and isolate 6-13-68 differ only with acid production from sucrose, salicin, melibiose, and raffinose. The latter three carbohydrates were either negative or not tested by Prevot (6) when he characterized S. ridiculosus.

**DISCUSSION**

The predominant microorganisms found in the large bowel of normal humans are the gram-negative, anaerobic, nonsporeforming bacilli of the family Bacteroidaceae. Zubrycki and Spaulding (9) showed that Bacteroides may outnumber coliforms by 100- or 1,000-fold in the feces of normal adults.

In two patients reported here, Bacteroides and Sphaerophorus were consistently present in feces. However, the Sphaerophorus population in the feces of patient B declined drastically after his first exposure to therapeutic TBI (150 r at 1.5 r/hr). The irradiation may have had some effect on the anaerobic flora, as the patient received no antibiotics or diet change during the study. This type of drastic change in the anaerobic population is the only one we have noticed in our microfloral studies of irradiated cancer patients. One year later the numbers of Bacteroides and Sphaerophorus in the same patient, after a similar exposure to TBI, were consistently high for 7 weeks.

The morphological and biochemical properties of the two Sphaerophorus isolates did not change during this 1-year period.

Although others have isolated Sphaerophorus
species from the large bowel of man, we believe this is the first report of their presence in the feces over a long time. The type of *Sphaerophorus* isolated in this study has more likely been isolated from the large bowel of man. However, owing to the lack of adequate methods for characterizing the gram-negative anaerobes, these isolates were probably lumped into the *Bacteroides* species. Moore et al. (5), Smith and Holdeman (8), and Finegold et al. (3) have recently standardized methods for characterizing and differentiating species of *Bacteroides, Sphaerophorus,* and *Fusobacterium* and, thus, have eased the task of identifying this medically important group of microorganisms. Moore et al. (5) have placed *Sphaerophorus* and *Fusobacterium* strains into one species, *Fusobacterium,* on the basis of butyric acid production from peptone or glucose. Except for a few differences in carbohydrate fermentation, our isolate most closely resembles *Fusobacterium ridiculosum* (5). However, on the basis of guanine-cytosine ratios, as determined in W. E. C. Moore's laboratory, isolate 6-13-68 resembles *Fusobacterium mortiferum,* which is the same as *Sphaerophorus freundii,* ATCC strain 9817 (W. E. C. Moore, personal communication). On comparing *S. freundii* with isolate 6-13-68, we found differences in hemolysis, hemagglutination, nitrate reduction (Table 4), and acid production from maltose and sucrose (Table 5). Prevot (7) described an *S. mortiferus,* an obligate serophile which produces acid from sucrose, mannitol, and sorbitol. Isolate 6-13-68 grows without blood or serum and fails to produce acid from sucrose, mannitol, and sorbitol.

**ACKNOWLEDGMENTS**

This investigation was supported by the U.S. Army Research and Development Command 8306 and was carried out at the Medical Division, Oak Ridge Associated Universities, under contract with the U.S. Atomic Energy Commission.

**LITERATURE CITED**

1. Deasor, B. S. 1967. Cultivation of anaerobic intestinal bacteria. J. Pathol. Bacteriol. 94:417-427.
2. Eggerth, A. H., and B. H. Gagnon. 1933. The *Bacteroides* of human feces. J. Bacteriol. 25:389-413.
3. Finegold, S. M., A. B. Miller, and D. J. Posnick. 1965. Further studies on selective media for *Bacteroides* and other anaerobes. Ernahrungsforschung 10:517-528.
4. Lewis, K. H., and L. F. Retger. 1940. Non-sporeulating anaerobic bacteria of the intestinal tract. I. Occurrence and taxonomic relationships. J. Bacteriol. 40:287-307.
5. Moore, W. E. C., E. P. Cato, C. S. Cummins, L. V. Holde-
man, J. L. Johnson, R. M. Smibert, and L. DS. Smith. 
1969. Outline of clinical methods in anaerobic bacteriology. 
The Virginia Polytechnic Institute Anaerobe Laboratory, 
Blacksburg, Va.
6. Prevot, A. R. 1948. Actinomycétale anaérobie stricte nouvelle: 
*Sphaerophorus ridiculosus* n. sp. Ann. Inst. Pasteur (Paris) 
75:387–389.
7. Prevot, A. R. 1966. Manual for the classification and deter-
mination of the anaerobic bacteria, p. 142. Lea and Febiger, 
Philadelphia.
8. Smith, L. DS., and L. V. Holdeman. 1968. The pathogenic 
anaerobic bacteria. Charles C Thomas, Publisher, Springfield, 
Ill.
9. Zubrycki, L., and E. H. Spaulding. 1962. Studies on the sta-
bility of the normal human fecal flora. J. Bacteriol. 83:968–
974.