Anaerobic gram-negative faecal flora in patients with Crohn’s Disease and healthy subjects

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The anaerobic gram-negative faecal flora of five patients with Crohn’s Disease (CD) was identified and compared with that of healthy subjects. For isolation and cultivation of the anaerobic gram-negative rods a non-selective medium was used. There were no significant differences in numbers of Bacteroides and Fusobacterium spp. between patients with CD and healthy subjects. However, the numbers of the “Bacteroides fragilis” group were significantly higher in patients than in controls. The high numbers of the “B. fragilis” group in the faeces of patients were particularly due to B. vulgatus which was 6 times more frequent in patients than in healthy subjects. This indicated that B. vulgatus was responsible for the higher numbers of anaerobic gram-negative rods in the faecal flora of patients with CD.

INTRODUCTION

The faecal flora of patients with CD has been found to differ from the flora of healthy subjects (Wensinck, 1975, 1976; Wensinck et al., 1981). Patients with CD have higher numbers of anaerobic gram-negative rods and anaerobic gram-positive coccoid rods (Eubacterium, Coprococcus and Peptostreptococcus). Ileocecal resection and duration and severity of disease had no effect on flora composition, which suggests that the abnormal flora does not merely result from the inflammatory process, but is likely to be the resident intestinal flora of patients suffering from CD (Wensinck et al., 1981). In the present study, the anaerobic gram-negative flora in patients with CD was identified and compared with those in healthy subjects.
It is usual to isolate *Bacteroides* on a selective medium. Preliminary studies, however, showed that addition of kanamycin, vancomycin or bile to the media to suppress the growth of gram-positive bacteria (Finegold et al., 1974; Goldberg et al., 1977; Cummings et al., 1978; Duerden, 1980; Krook et al., 1981) resulted in a 25–50% loss of *Bacteroides* species. Therefore, we used non-selective media for cultivation and subsequent identification of the gram-negative flora in both healthy subjects and patients with CD.

**Materials and Methods**

**Subjects**
Five healthy volunteers aged 23–42 years (median 35) and five patients with CD aged 41–52 years (median 47) were studied. The diagnosis of CD was established according to usual criteria (Lennard-Jones et al., 1968; Kirsner, 1975). The principal site of inflammation was the ileum. The patients did not take medicine during the last three months, had no more than two formed stools a day, without blood and did not have other diseases. Consequently, all patients had inactive disease as judged by a CD activity index (Best et al., 1976) of < 150. One sample of faeces from every subject was examined.

**Media**
The dilution-fluid consisted of trypton (Oxoid, Basingstoke, England) 0.5% (w/v), casiton (Difco, Detroit, USA) 0.5%, glucose 0.5%, NaCl 0.5%, K$_2$HPO$_4$·3H$_2$O 0.3%, cysteine-HCl 0.05%, resazurine 0.0002%, agar (Difco) 0.05%; the pH was adjusted to 7.2–7.3. In anaerobic culture flasks (Wensinck and Ruseler-van Embden, 1971) a non-selective medium, Schaedler Broth (Oxoid) with 0.0002% resazurine and 2% agar was used (pH 7.6). Schaedler Broth with resazurine was used for the estimation of fermentation end-products. All media were sterilized by autoclaving for 15 min at 121°C.

**Isolation**
Stools were diluted and plated according to the methods of Wensinck and Ruseler-van Embden (1971) and Wensinck et al. (1981) with minor changes. Within two h of passage, samples of 10 g were transferred into 190 ml of dilution fluid and homogenized ("Stomacher" Lab-Blender 400). From this suspension a 2.10$^6$ fold dilution was prepared of which 0.1 and 0.2 ml were spread out on the solid medium in anaerobic culture flasks and incubated for three days at 37°C. Flasks with 80–200 colonies were used for counting and identification. All colonies grown in the flasks were picked up and gram-stained; the gram-negatives were subcultured on the same medium. Subculturing was not always successful. Approximately 10% of the colonies failed to grow on the solid medium in the flasks and 10–20% did not grow in