Prevalence of *Bacteroides* and *Prevotella* spp. in ulcerative colitis

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The resident bacterial flora of the large intestine has become increasingly recognized as an essential component in the pathogenesis of ulcerative colitis (UC). However, it is still not known whether the bacterial flora in general or certain bacterial species of the intestinal microbial flora contribute to the pathogenesis of the disease. In order to investigate the composition of the mucosa-associated microbial flora in UC, mucosal tissue samples from patients with active UC and from control subjects with non-inflammatory conditions were analysed and compared. To cover the whole spectrum of intestinal bacteria and to circumvent the known bias introduced by culture-based techniques, comparative 16S rRNA gene sequence analysis was used to determine the bacterial composition in the mucosal tissue samples. The investigation revealed an abundance of sequences from *Bacteroides* spp. and *Prevotella* spp. in the mucosal tissue of patients with UC compared with individuals showing no signs of disease. The higher incidence of populations of members of the *Bacteroidetes* in UC suggests that these may have an influence on the pathogenesis of the disease.

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INTRODUCTION

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) characterized by strong activation of the mucosa-associated immune system due to a complex interaction of genetic, immunological and environmental factors. It is established that the composition of the intestinal microbial flora has an impact on host immunity and influences the course of mucosal inflammation (Hooper & Gordon, 2001; Hooper *et al.*, 2001). Various studies implicate a role for resident intestinal bacteria in the pathogenesis of UC (Mishina *et al.*, 1996; Papadakis & Targan, 1999). Observations such as a high antibody response to bacterial antigens in patients with IBD (Macpherson *et al.*, 1996) and the therapeutic effect of antibiotics lend further support to the hypothesis of a bacterial contribution to the pathogenesis of IBD (Chapman *et al.*, 1986; van Kruiningen, 1995). The necessity of bacteria for the initiation and perpetuation of chronic inflammation has been demonstrated in various animal models (Blumberg *et al.*,

1999; Sellon *et al.*, 1998; Waidmann *et al.*, 2003). Studies of T-cell receptor-deficient mice indicate that it is not the presence of intestinal microflora per se, but rather the incidence of certain bacteria that seems to be of particular importance (Dianda *et al.*, 1997). Experiments in rats with induced experimental colitis suggest the involvement of anaerobic bacteria (Garcia-Lafuente *et al.*, 1997; Rath *et al.*, 1999).

The above-mentioned studies clearly demonstrate that intestinal bacteria play a crucial role in the pathogenesis of IBD. Therefore, investigation of the mucosa-associated bacterial flora constitutes an important pre-requisite in order to be able to address the question of whether the enteric flora per se or a singular bacterial species contributes to IBD pathogenesis (Fiocchi, 1998).

The large intestine of humans harbours up to 10¹⁴ bacteria comprising an estimated 400–500 species (Berg, 1996), of which most indigenous microbial species remain unknown (Eckburg *et al.*, 2005; Suau *et al.*, 1999). The identification of bacteria on the basis of their 16S rRNA gene sequences using molecular techniques overcomes the limitations of culture-based techniques and represents a state-of-the-art tool for the culture-independent analysis of complex microbial communities (Amann *et al.*, 1995).

Abbreviations: DIG, digoxigenin; IBD, inflammatory bowel disease; UC, ulcerative colitis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences determined in this study are AJ812137–AJ812212.

The aim of this study was to analyse and compare the bacterial composition of the mucosa-associated microbial flora in mucosal tissue from patients with active UC and patients without IBD to determine the potential association of particular bacterial populations with intestinal inflammation in UC.

METHODS

Clinical specimens. This study was approved by the ethics committee of the Medical Faculty of the Technical University Dresden. All patients undergoing colonoscopy consented to providing an extra biopsy tissue for study purposes. Colonic biopsy samples were obtained from six patients in whom colonoscopy was done in the course of routine clinical care. Two individuals (patient P128 and P2110) underwent colonoscopy for cancer surveillance. They had no gastrointestinal complaints and revealed non-inflammatory conditions. The four IBD patients included in this study (patients P67, P77, P118 and P178) were in an active stage of chronic UC with mucosal inflammation, which was obvious from the clinical status, endoscopy and histology. Two of the patients (P67 and P118) revealed complete mucosal inflammation of the colon during colonoscopy. None of the patients included in the study had received antibiotic therapy or other drugs known to influence the faecal flora within the 6 months prior to the endoscopic investigation. Patients P77 and P118 received therapy with mesalazine (5-aminosalicylic acid); no therapy was administered to the other patients. None of the patients included in this study received a special diet.

The colonoscopy preparation using Klean-Prep (Norgine) followed an evidence-based procedure (Ell *et al.*, 2003) and was the same for all of the patients included in this study: patients started with a first lavage (2 l) the night before the endoscopy and received a second lavage (2–3 l) supervised by a hospital nurse on the endoscopy ward the following morning.

Biopsies were collected with a flexible videocoloscope (CFQ160AI; Olympus) and spiked biopsy forceps (FB24U; Olympus). In patients P77 and P178, biopsies were collected from macroscopically inflamed mucosal sites, as well as from adjacent mucosa that appeared normal macroscopically. For each patient, three to four biopsy samples from each location were pooled to minimize sampling bias. The sampled biopsies were immediately frozen in liquid nitrogen and stored at -80°C until extraction of nucleic acids.

Amplification of 16S rRNA genes. Chromosomal DNA was extracted from bacterial cultures and gastrointestinal biopsy specimens using the DNeasy Tissue kit (Qiagen) following the manufacturer's recommendations. The 16S broad-range primers 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-GGCGG-GWGTGTACAAGGC-3') were used to amplify almost-complete 16S rRNA genes from the mixed template preparations (Marchesi *et al.*, 1998). Amplification was performed on a GeneAmp PCR System 2400 Thermal Cycler (PE Applied Biosystems). In brief, serial dilutions of the extracted DNA were used in 50 μl PCRs containing 1 $\times$ GeneAmp PCR buffer comprising 1.5 mM MgCl_2 (PE Applied Biosystems), 200 μM each dNTP, 1 μM each primer and 1.25 U AmpliTaq DNA Polymerase LD. PCR cycle conditions comprised an initial denaturation step of 2 min at 95°C , followed by 30 cycles of denaturation for 60 s at 95°C , annealing for 60 s at 55°C and extension for 2 min at 72°C , with a single final extension step of 10 min at 72°C . Negative PCR controls containing no DNA were run in parallel. Amplified PCR products were

analysed by 1.5% agarose gel electrophoresis and purified using the QIAquick PCR Purification kit (Qiagen) following the manufacturer's specifications.

Cloning of PCR products and colony-blot analysis. Purified PCR products were cloned using the TOPO TA Cloning kit (Invitrogen) as indicated by the manufacturer. From each cloning reaction, 1000 recombinant clones were plated on to Luria–Bertani agar supplemented with 0.1 mg ampicillin ml^{-1} .

To quantify the number of recombinant clones representing the 16S rRNA gene from *Bacteroides* spp. or *Prevotella* spp., colony hybridization using the 5' digoxigenin (DIG)-labelled oligonucleotide probe BAC303 (5'-CCATGTGGGGGACCTT-3') (Manz *et al.*, 1996) was performed. Bacterial colonies were transferred to positively charged nylon membrane discs (Millipore) and lysed according to standard protocols. In brief, two layers of Whatman 3MM paper were soaked for each of the following: denaturation solution (0.5 M NaOH, 1.5 M NaCl), neutralization solution (1.5 M NaCl, 1.0 M Tris/HCl, pH 7.5) and $2 \times$ SSC buffer (300 mM NaCl, 30 mM sodium citrate, pH 7.0). Membrane discs were placed colony-side up on filter paper soaked with denaturation solution for 15 min, followed by blotting of the membrane discs briefly on Whatman 3MM paper. The discs were then placed for 15 min on to prepared filter paper soaked in neutralization solution. After blotting of the membrane discs on Whatman 3MM paper, they were placed on to prepared filter paper soaked with $2 \times$ SSC buffer for 10 min. Fixation of the DNA to the nylon membranes was performed by baking the dried membrane discs for at least 30 min at 80°C .

After immobilization of the DNA, proteinase K treatment was performed to remove cellular and agar debris. For this purpose, the membrane discs were placed on to a clean piece of aluminium foil, 0.5 ml proteinase K solution (2 mg ml^{-1}) was added and evenly distributed, and the discs were incubated at 37°C for 1 h. The membrane discs were blotted between Whatman 3MM paper and pressure was applied by passing a bottle over the area before the upper filter paper, with the debris sticking to it, was gently pulled off.

Hybridization was performed in a hybridization oven (Biometra) using roller bottles. Membrane discs were placed in a roller bottle and pre-hybridized with 60 ml hybridization solution without oligonucleotide probe [$5 \times$ SSC, 0.1% (w/v) *N*-laurylsarcosine, 0.02% (w/v) SDS, 1.0% (w/v) blocking reagent (Boehringer Mannheim)] for 1 h at 43°C . The DIG-labelled oligonucleotide probe BAC303 was denatured by boiling for 5 min at 95 – 100°C and subsequently diluted in pre-hybridization solution to a concentration of 10 pmol ml^{-1} . Hybridization was performed using 6 ml of this hybridization solution at 43°C . After 2 h, the membranes were washed twice in 50 ml washing buffer [$5 \times$ SSC, 0.1% (w/v) SDS] for 15 min at the hybridization temperature. Detection of DIG-labelled probe was conducted using anti-DIG antibodies coupled to alkaline phosphatase (DIG Luminescent Detection kit; Boehringer Mannheim) as indicated by the manufacturer. Chemiluminescence was determined by exposure of membranes to X-ray films (X-Omat AR; Kodak).

16S rRNA gene analysis. The 16S rRNA gene insert of recombinant clones was amplified and sequenced as described elsewhere (Schuppler *et al.*, 2004). To determine the approximate phylogenetic relationships, the partial 16S rRNA gene sequences were subjected to a BLAST search against the EMBL and GenBank nucleotide sequence databases as described previously (Schuppler *et al.*, 1995). By using >95 and >97 % sequence similarity, respectively, to delimit a genus and a species, the sequences were assigned to the respective bacterial phylotypes.

RESULTS

Comparison of the mucosa-associated bacterial flora in patients with UC and individuals without IBD

In general, the 16S rRNA gene clone libraries harboured well-known members of the human gastrointestinal tract, as well as putative new, hitherto-uncultivated bacteria. Based on the results of the BLAST search, the sequences were assigned to the major bacterial taxa *Actinobacteria*, '*Bacilli*', *Bacteroidetes*, '*Clostridia*', '*Fusobacteria*' and *Proteobacteria*. Table 1 shows the relative abundance of the different phylogenetic groups represented by the clone libraries. The majority of the recovered sequences belonged to members of the *Bacteroidetes* and '*Clostridia*'. All 16S rRNA gene libraries contained members of the *Bacteroidetes* with *Bacteroides vulgatus* as the major representative. Sequences from other species such as *Bacteroides caccae*, *Bacteroides distasonis*, *Bacteroides fragilis*, *Bacteroides merdae*, *Bacteroides ovatus*, *Bacteroides putredinis*, *Bacteroides thetaiotaomicron*, *Bacteroides uniformis* and the recently described *Bacteroides massiliensis* (Fenner *et al.*, 2005) were also frequently identified. There was one exception: the libraries from patient P178 were dominated by a 16S rRNA gene sequence from a hitherto-unknown member of the *Bacteroidetes*. Several clones revealed high sequence similarity to previously described sequences from hitherto-uncultured bacteria such as the uncultured bacterium adhufec367 reported by Suau *et al.* (1999).

'*Clostridia*' were also harboured by all libraries and were mainly represented by sequences from hitherto-unknown members. Known species such as *Clostridium aerofaciens*, *Clostridium celatum*, *Clostridium cocleatum*, *Clostridium innocuum*, *Clostridium paraputrificum*, *Clostridium ramosum*, *Clostridium spiroforme*, *Ruminococcus bromii*, *Ruminococcus gnavus*, *Ruminococcus lactaris*, *Ruminococcus torques* and *Faecalibacterium prausnitzii* occurred in low numbers.

For the *Proteobacteria*, most sequences were derived from the *Gammaproteobacteria* and were closely related to previously cultured bacteria including *Acinetobacter johnsonii*, *Haemophilus parainfluenzae*, *Stenotrophomonas maltophilia* and *Escherichia coli*, which was the dominant species in most libraries of the *Gammaproteobacteria*. However, unlike the *Bacteroidetes* and '*Clostridia*', not all libraries harboured sequences from members of the *Enterobacteriaceae*. Members of the *Alpha*- and *Betaproteobacteria* were found only sporadically in the libraries and represented known species.

Beside sequences with high similarities to database sequences, the libraries also harboured numerous sequences revealing only weak homologies (<95%). A considerable fraction of sequences derived from hitherto-uncultivated bacteria that are known only by their 16S rRNA gene sequences recovered using molecular approaches from gut

samples of humans (Eckburg *et al.*, 2005; Hold *et al.*, 2002; Suau *et al.*, 1999; Wang *et al.*, 2003) or other mammals (Leser *et al.*, 2002; Pryde *et al.*, 1999; Salzman *et al.*, 2002). Sequences from recently identified bacteria, such as a butyrate-producing bacterium cultured from a human gut sample (Barcenilla *et al.*, 2000), were also found.

Comparison of the 16S rRNA gene clone libraries from the UC patients and the healthy individuals did not determine sequences that occurred exclusively in all libraries from the patients and not in the control group. However, an obvious difference was the abundance of members of the *Bacteroidetes* in the libraries from the UC patients. As demonstrated in Table 1, all clone libraries from UC patients were characterized by a predominance of sequences from strict anaerobes belonging to the *Bacteroides*–*Prevotella* cluster of the *Cytophaga*–*Flavobacter*–*Bacteroides* group. Consequently, sequences from members of the *Bacteroidetes* represented 50–82% of the total number of analysed clones in the libraries from UC patients, whereas the 16S rRNA gene libraries from the non-IBD patients P128 and P2110 contained 16 and 25%, respectively.

Colony hybridization of 16S rRNA gene clone libraries

Sequence analysis of 60–80 recombinant clones from each of the established 16S rRNA gene clone libraries revealed a predominance of members of the *Bacteroidetes* in the mucosal biopsy samples from UC patients. In order to verify this observation, 1000 recombinant clones from the clone libraries of UC patient P178 and non-IBD patient P2110 were analysed by colony-blot hybridization. Oligonucleotide probe BAC303 was used for specific detection of clones containing 16S rRNA gene sequences from *Bacteroides* spp. or *Prevotella* spp. The specificity of BAC303 was demonstrated by the fact that all recombinant clones previously identified as *Bacteroides* or *Prevotella* sequences gave a positive hybridization signal with this probe. In contrast, none of the previously analysed clones harbouring sequences from other bacteria resulted in a positive hybridization signal. Due to the specificity of probe BAC303, which was restricted to sequences of the genera *Bacteroides* and *Prevotella*, colony hybridization resulted in slightly lower counts compared with the values for members of the *Bacteroidetes* determined by sequence analysis of the respective clone libraries. However, as is apparent from Table 2, the relative proportions proved to be consistent with the results from the comparative 16S rRNA gene analysis, confirming the abundance of *Bacteroides* spp. and *Prevotella* spp. (45 and 56% for the inflamed and non-inflamed UC areas, respectively) in the clone libraries from UC patient P178 compared with the non-IBD patient P2110 (14%).

Comparison of sites of inflammation and sites without apparent inflammation

Colonoscopy revealed areas of mucosal inflammation and areas without apparent inflammation of the mucosa for

Table 1. Phylogenetic distribution of 16S rRNA gene sequences from biopsies of UC patients and healthy controls

Numbers in parentheses are percentages.

Taxon	Patients with UC				Healthy controls	
	P77 colitis (n = 128)	P178 colitis (n = 124)	P67 pancolitis (n = 73)	P118 pancolitis (n = 66)	P128 no IBD (n = 54)	P2110 no IBD (n = 53)
Actinobacteria	—	—	—	—	1 (2)	—
<i>Bifidobacterium pseudocatenulatum</i>	—	—	—	—	1	—
'Bacilli'	—	—	—	1 (2)	1 (2)	—
<i>Enterococcus faecalis</i>	—	—	—	1	—	—
<i>Streptococcus salivarius</i>	—	—	—	—	1	—
Bacteroidetes	76 (59)	71 (57)	60 (82)	33 (50)	9 (16)	13 (25)
<i>Bacteroides caccae</i>	—	4	—	—	—	2
<i>Bacteroides distasonis</i>	5	4	—	2	2	—
<i>Bacteroides fragilis</i>	1	1	—	7	—	—
<i>Bacteroides massiliensis</i>	16	—	9	—	—	—
<i>Bacteroides merdae</i>	—	4	—	—	—	—
<i>Bacteroides ovatus</i>	—	2	—	5	2	1
<i>Bacteroides putredinis</i>	—	—	—	—	2	—
<i>Bacteroides thetaiotaomicron</i>	—	—	—	—	—	—
<i>Bacteroides uniformis</i>	1	—	—	—	—	—
<i>Bacteroides vulgatus</i>	46	1	50	17	—	9
Unidentified <i>Bacteroides</i> sp.	7	13	1	2	2	1
Unidentified <i>Prevotella</i> sp.	—	42	—	—	1	—
'Clostridia'	40 (31)	46 (37)	11 (15)	13 (20)	40 (74)	10 (19)
<i>Collinsella aerofaciens</i>	—	3	—	—	3	—
<i>Clostridium celatum</i>	—	—	—	—	—	5
<i>Clostridium cocleatum</i>	—	1	—	—	—	—
<i>Clostridium innocuum</i>	1	—	—	—	1	—
<i>Clostridium paraputrificum</i>	—	1	—	—	—	1
<i>Clostridium ramosum</i>	6	2	—	1	—	—
<i>Clostridium spiroforme</i>	—	—	—	—	2	—
<i>Clostridium</i> sp.	9	7	—	5	6	1
<i>Eubacterium</i> sp.	1	5	1	—	4	—
<i>Faecalibacterium prausnitzii</i>	3	12	—	—	1	—
<i>Ruminococcus bromii</i>	—	—	—	—	1	—
<i>Ruminococcus gnavus</i>	—	—	3	—	—	1
<i>Ruminococcus lactaris</i>	—	1	—	—	—	—
<i>Ruminococcus torques</i>	—	1	—	—	—	—
<i>Ruminococcus</i> sp.	8	2	3	1	3	—
Unidentified 'Clostridia'	12	11	4	6	19	2
'Fusobacteria'	—	—	—	2 (3)	—	—
<i>Fusobacterium nucleatum</i>	—	—	—	2	—	—
Alphaproteobacteria	—	5 (4)	—	—	1 (2)	1 (2)
<i>Phyllobacterium rubiacearum</i>	—	2	—	—	—	1
<i>Sphingomonas paucimobilis</i>	—	3	—	—	1	—
Betaproteobacteria	—	—	—	—	1 (2)	—
<i>Hydrogenoluteola thermophilus</i>	—	—	—	—	1	—
Gammaproteobacteria	12 (10)	2 (2)	2 (3)	17 (25)	1 (2)	29 (54)
<i>Acinetobacter johnsonii</i>	—	—	—	1	—	—
<i>Escherichia coli</i>	12	—	2	15	—	28
<i>Haemophilus</i> sp.	—	1	—	1	1	—
<i>Providencia</i> sp.	—	1	—	—	—	—
<i>Stenotrophomonas maltophilia</i>	—	—	—	—	—	1
<i>paraphrohaemolyticus</i>	—	—	—	—	—	—

Table 2. Comparison of the relative proportions of members of the *Bacteroidetes* determined by sequence analysis of 70 clones or by colony-blot hybridization of 1000 clones per 16S rRNA gene library

Values are the percentage of identified clones in the respective libraries.

Library	Sequence analysis (70 clones per library)	Colony-blot analysis (1000 clones per library)
P178k (UC inflamed area)	54	45
P178g (UC non-inflamed area)	61	56
P2110 (no IBD)	24	14

patients P77 and P178. Consequently, separate 16S rRNA gene libraries were established for these patients: one from inflamed mucosa and one from non-inflamed mucosa. The libraries from the different regions of patient P178 revealed a very similar bacterial composition and abundance (Table 3). Almost exclusively, they harboured sequences from members of the *Bacteroidetes* and '*Clostridia*', with only a few sequences representing other taxa. In contrast, the samples from patient P77 revealed differences in the bacterial composition of libraries from inflamed and non-inflamed mucosae. Both libraries harboured additionally sequences from *E. coli*, but revealed noticeable differences with respect to the qualitative and quantitative distribution of sequences from members of the *Bacteroidetes*, '*Clostridia*' and the *Enterobacteriaceae* (Table 3). According to the results from the UC patients showing complete mucosal inflammation of the colon, the libraries from inflamed mucosa as well as from non-inflamed mucosa revealed an abundance of members of the *Bacteroidetes*.

DISCUSSION

This study highlights the abundance of members of the genera *Bacteroides* and *Prevotella* in mucosal biopsy specimens from UC patients. It also suggests that the presence of a particular bacterial species or pathogen may not be associated with the disease. Mucosal biopsy specimens from patients suffering from UC and individuals showing no signs of IBD were investigated by a culture-independent 16S

rRNA gene approach. The resulting 16S rRNA gene clone libraries revealed a diverse microbiota, representing well-known members of the human gastrointestinal tract, as well as putative new sequences from hitherto-uncultivated species and novel phylotypes, which have also been reported from other 16S rRNA gene-based studies of human gastrointestinal samples (Eckburg *et al.*, 2005; Wang *et al.*, 2003). This observation demonstrates that the approach applied in this study was effective in gathering the broad spectrum of intestinal bacteria.

A comparison of results from the UC patients and non-IBD patients in this study did not lead to the identification of a specific sequence or group of sequences exclusively harboured in the samples from the UC patients. Therefore, it was not possible to relate certain bacterial species to the presence or absence of the colonic illness. This correlates well with reports from denaturing and temporal temperature gradient gel electrophoretic analyses of mucosal biopsies from healthy and diseased individuals (Seksik *et al.*, 2003; Zoetendal *et al.*, 2002). The generated profiles of the predominant bacterial communities appeared to be complex and unique for each individual, and no specific single band could be related to the presence or absence of colonic illness.

The most obvious difference in the mucosa-associated flora from the UC and non-IBD patients was the abundance of *Bacteroides* and *Prevotella* in the 16S rRNA gene libraries from the UC patients. It is well known that the quantitative distribution of sequences in 16S rRNA gene libraries does not necessarily reflect actual quantities of the respective bacterial populations in the original samples. However, all samples investigated in this study were processed equally in terms of sample collection, DNA isolation procedure, PCR amplification and the kits used for establishing the clone libraries. Therefore, the predominance of sequences from members of the *Bacteroidetes* in the libraries from the UC patients points to an abundance of members of this phylum in the mucosa of the UC patients compared with the non-IBD patients.

Although care was taken that all biopsy samples included in this study were obtained and investigated in a standardized manner, it must be taken into account that the mucosal flora is exposed to factors such as purgatives, laxatives,

Table 3. Comparison of the composition of 16S rRNA gene libraries from inflamed and macroscopically normal-looking mucosae

Values are the percentage of identified clones for the respective libraries.

Taxon	P77 inflammation		P178 inflammation	
	Yes	No	Yes	No
<i>Bacteroidetes</i>	74	42	54	61
' <i>Clostridia</i> '	10	53	40	33
<i>Enterobacteriaceae</i>	16	5	0	0
Other bacteria	0	0	6	6

antimicrobial agents and diet, which may affect the residual flora prior to colonoscopy. We tried to avoid this by accurate choice of the individuals included in this study and by applying a standardized and evidence-based preparation procedure (Ell *et al.*, 2003). None of the individuals included in the study received antimicrobial therapy within the 6 months prior to colonoscopy and none of the patients received a special diet that would provoke changes in the faecal flora. Two patients (P77 and P118) received therapy with mesalazine; no therapy was administered to any of the other patients included in this study. Despite reports that the preparation procedure mainly affects the luminal flora, whilst the mucosa-associated flora seems to be less influenced by such procedures (Marks *et al.*, 1979), a potential influence on the residual flora could not be excluded totally. However, all patients included in this study received the same preparation prior to colonoscopy.

Increased counts for members of the *Bacteroidetes* in the colonic mucosa of IBD patients have also been reported from culture-based investigations. Ruseler-van Embden & Both-Patoir (1983) observed an increase in predominantly anaerobic flora, especially *Bacteroides* spp., in active Crohn's disease, whilst Hartley *et al.* (1992) isolated a higher level of *Bacteroides* spp. from patients with active UC, whether at first onset or upon release, than from patients with inactive disease. Swidsinski *et al.* (2002) reported a preferential increase in *Bacteroides* spp. and *E. coli* in IBD patients. Similar to the results from this study, the highest mean concentrations for *Bacteroides* spp. were reported for patients with UC or Crohn's disease. Interestingly, the abundance of members of the *Bacteroidetes* in the mucosa of UC patients was not restricted to inflamed mucosal sites. This is in concordance with the results from this study, which revealed high levels of members of the *Bacteroidetes* for both inflamed and non-inflamed mucosal areas. Furthermore, Swidsinski *et al.* (2002) found no significant differences in the bacterial spectrum between inflamed and non-inflamed regions of the mucosa, but demonstrated higher numbers of bacteria in the mucosa-associated flora of UC patients compared with healthy individuals in correlation to the severity of the disease. The observation of low numbers of members of the *Bacteroidetes* in the mucosal tissue from the healthy controls in this study is also consistent with other reports on the composition of the mucosal flora of healthy humans (Ott *et al.*, 2004).

Evidence for a pro-inflammatory role for members of the *Bacteroidetes* also comes from animal models for IBD where *Bacteroides* spp. appear to play a pivotal role in the induction of colitis (Onderdonk *et al.*, 1983; Rath *et al.*, 1996). The incidence of members of the *Bacteroidetes* would also explain the therapeutic response to metronidazole in IBD patients and animal models for IBD. Metronidazole is particularly active against strictly anaerobic bacteria and it is reported that treatment of active disease in IBD patients with metronidazole results in complete or partial clinical remission and also prevents recurrence after ileal resection (Greenbloom *et al.*, 1998; Prantera *et al.*, 1998; Rutgeerts *et al.*, 1995).

Furthermore, the protective effect of metronidazole in experimental UC has been demonstrated and suggests that anaerobic bacteria play a role in the initial events of experimental colitis in the guinea pig model (Onderdonk *et al.*, 1978).

The mechanisms by which *Bacteroides* spp. may contribute to the chronic inflammatory process are chiefly unknown. Breeling *et al.* (1988) reported an association of outer-membrane antigens from *B. vulgatus* with carrageenan-induced colitis in guinea pigs. Another possible mechanism by which members of the *Bacteroidetes* probably contribute to the pathogenesis of colitis is by the production of mucin-degrading sulphatases. Elevated levels of bacterial mucin-desulphating sulphatases have been reported for patients suffering from active UC (Tsai *et al.*, 1995). Furthermore, the existence of enzymes that will partially desulphate mucins has been demonstrated for *B. thetaiotaomicron*, *B. fragilis* (Tsai *et al.*, 1992) and for *Prevotella* spp. (Wright *et al.*, 2000), suggesting that members of the *Bacteroidetes* could contribute to chronic inflammation by an impairment of the barrier function of the epithelial cell layer.

E. coli is also frequently encountered as potentially being involved in the pathogenesis of UC, and several studies on animal models for IBD support an association of *E. coli* with the disease (Schuppler *et al.*, 2004; Waidmann *et al.*, 2003). However, the detection of sequences from *E. coli* in the samples from the non-IBD patients together with the observed absence of *E. coli* in several samples from UC patients does not substantiate a primary role for *E. coli* in human UC. *Mycobacterium avium* subsp. *paratuberculosis*, *Helicobacter* spp., *Listeria monocytogenes* and several other bacterial pathogens have also often been related to UC. In particular, *Campylobacter*, *Salmonella*, *Shigella* and *Yersinia* have been reported as responsible for an inflammatory relapse in UC (Campirei & Gionchetti, 2001), but the 16S rRNA gene libraries established in this study did not contain sequences from known bacterial pathogens.

In conclusion, the results from this study point to an increase in the abundance of members of the *Bacteroidetes* in the colonic mucosa of UC patients. This is an important finding, as it suggests that certain bacterial populations are altered in active UC and therefore may play a role in the pathogenesis of the disease. However, the observation of an increase in members of the *Bacteroidetes* in the mucosal flora of IBD patients in this and other studies does not necessarily establish an aetiological connection. Therefore, it cannot be deduced whether the increase in anaerobes in the mucosal flora is causative or a consequence of the disease and whether specific members of the *Bacteroidetes* play a primary role in initiation of the disease. Further studies of an extended number of patients are needed to determine to what extent the changes reported in this study are primary or secondary to alterations of the mucosal microenvironment. In order to investigate the aetiological mechanisms of alterations in the bacterial microflora, longitudinal studies may provide important information.

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