Intestinal Bacteria and Ulcerative Colitis

J. H. Cummings*, G. T. Macfarlane, and S. Macfarlane

Department of Molecular and Cellular Pathology,
University of Dundee, Ninewells Hospital Medical School,
Dundee, DD1 9SY, UK

Abstract

Convincing evidence from both animal models and the study of patients with ulcerative colitis (UC) implicates the intestinal microflora in the initiation and maintenance of the inflammatory processes in this condition. Despite this, no specific pathogen has been identified as causal and the disease is widely believed to occur as the result of a genetically determined, but abnormal immune response to commensal bacteria. When compared with healthy people, UC patients have increased levels of mucosal IgG directed against the normal microflora. Studies of mucosal bacterial populations in UC indicate that there may be increased numbers of organisms, but reduced counts of "protective" bacteria such as lactobacilli and bifidobacteria. In animal models of colitis, antibiotics, particularly metronidazole, clindamycin, ciprofloxacin and the combination of vancomycin/impinemem protect against UC, especially if given before the onset of inflammation. These antibiotics target anaerobes and some Gram-positive organisms such as enterococci. However, antibiotic use in more than a dozen randomised control trials has been very disappointing, probably because we do not know which species to target, when to give the antibiotics, for how long and in what combinations. Surprisingly, therefore, there is a consistent benefit in the small number of studies reported of probiotics to manage UC and pouchitis. There is scope for more work in this area focussing on the mucosal microflora, its interactions with the gut immune system, its metabolic properties and the potential ways of modifying it.

Introduction

Microorganisms cover the surface of the large bowel mucosa and bacterial cell densities in adjacent lumenal contents are around 10^{12} per gram. Most of us tolerate this complex metabolically active and antigenic microflora, but in approximately two per 1000 of adults living in industrialised western countries (Mayberry *et al.*, 1989; Montgomery *et al.*, 1998; Loftus *et al.*, 2000) an intense inflammation develops in the mucosa, associated with bloody diarrhoea, urgency to defecate and general malaise, that is not associated with known pathogens.

*For correspondence. Email h.cowper@dundee.ac.uk;
Tel. +44(0)1382 632425; Fax. +44(0)1382633952.

Ulcerative colitis (UC) is one of the two major forms of idiopathic inflammatory bowel disease (IBD). It is an acute and chronic disabling condition treated primarily with drugs that suppress inflammation. Antibiotics are of limited use, despite overwhelming evidence that bacteria are involved in a process whereby genetically determined but abnormal mucosal immune responses occur to the microflora (Campieri and Gionchetti, 2001; Linskens *et al.*, 2001; Farrell and Peppercorn, 2002; Farrell and La Mont, 2002). UC, unlike Crohn's disease (CD), occurs only in the large bowel, where bacterial numbers are several orders of magnitude greater than in the rest of the gut, and where the rate of passage of material is characterised by the slow movement of digestive materials (Eve, 1966; Metcalf *et al.*, 1987; Cummings *et al.*, 1992). However, the human large intestine contains only about 200 grams of contents (Cummings *et al.*, 1990), and in such a large organ with a surface area of between 1 and 2 m^2, that is often distended with gas, this means that parts of the mucosal surface will not be in contact with the lumenal microflora for some hours each day. This is especially so for the sigmoid and rectal regions, which are emptied after defecation, yet it is these areas where UC always commences. This points to an adherent mucosal microflora rather than lumenal bacteria as the causal agents.

Other circumstantial evidence for the microflora being essential in UC comes from animal models (Elson *et al.*, 1995; Fiocchi, 1998), the study of serum and mucosal antibodies (Macpherson *et al.*, 1996; Hooper *et al.*, 2001), characterisation of the mucosal bacterial communities in patients with active disease, and recently, the use of probiotic bacteria in treatment (Kruis, *et al.*, 1999; Rembacken *et al.*, 1999).

Bacteria Associated with UC

Many bacteria evoke an acute inflammatory response in the host gut mucosa. The principal organisms involved are toxigenic, adherent or invasive to the gut epithelium, and include pathogenic *Escherichia coli*, as well as species belonging to the genera *Yersinia*, *Shigella, Salmonella, Campylobacter, Clostridium* and *Aeromonas* (Cohen and Giannella, 1991; Macfarlane and Gibson, 1995). The clinical effects of these infections are usually acute rather than chronic, and the pathogenic mechanisms involved and host responses have been well studied. However, the role of bacteria in other, more chronic forms of gut disease such as antibiotic-associated colitis, or UC and CD is less clear.

Species that are part of the colonic microflora are believed to be involved in both the initiation and maintenance stages of UC. In early microbiological studies, a variety of bacteria including *Streptococcus mobilis*, fusobacteria, and shigellas received attention as being involved in IBD (Onderdonk, 1983), largely because these organisms are able either to penetrate the gut epithelium, or cause a similar spectrum of pathology in experimental

Further Reading

- **MALDI-TOF Mass Spectrometry in Microbiology**
  Edited by: M Kostrzewa, S Schubert (2016)
  www.caister.com/malditof

- *Aspergillus* and *Penicillium* in the Post-genomic Era
  Edited by: RP Vries, IB Gelber, MR Andersen (2016)
  www.caister.com/aspergillus2

- **The Bacteriocins: Current Knowledge and Future Prospects**
  Edited by: RL Dorit, SM Roy, MA Riley (2016)
  www.caister.com/bacteriocins

- **Omics in Plant Disease Resistance**
  Edited by: V Bhadauria (2016)
  www.caister.com/opdr

- **Acidophiles: Life in Extremely Acidic Environments**
  Edited by: R Quatrini, DB Johnson (2016)
  www.caister.com/acidophiles

- **Climate Change and Microbial Ecology: Current Research and Future Trends**
  Edited by: J Marxsen (2016)
  www.caister.com/climate

- **Biofilms in Bioremediation: Current Research and Emerging Technologies**
  Edited by: G Lear (2016)
  www.caister.com/biorem

- **Microalgae: Current Research and Applications**
  Edited by: MN Tsaloglou (2016)
  www.caister.com/microalgae

- **Gas Plasma Sterilization in Microbiology: Theory, Applications, Pitfalls and New Perspectives**
  Edited by: H Shintani, A Sakudo (2016)
  www.caister.com/gasplasma

- **Virus Evolution: Current Research and Future Directions**
  Edited by: SC Weaver, M Denison, M Roossinck, et al. (2016)
  www.caister.com/virusevol

- **Arboviruses: Molecular Biology, Evolution and Control**
  Edited by: N Vasilakis, DJ Gubler (2016)
  www.caister.com/arbo

- *Shigella*: **Molecular and Cellular Biology**
  Edited by: WD Picking, WL Picking (2016)
  www.caister.com/shigella

- **Aquatic Biofilms: Ecology, Water Quality and Wastewater Treatment**
  Edited by: AM Romaní, H Guasch, MD Balaguer (2016)
  www.caister.com/aquaticbiofilms

- **Alphaviruses: Current Biology**
  Edited by: S Mahalingam, L Herrero, B Herring (2016)
  www.caister.com/alpha

- **Thermophilic Microorganisms**
  Edited by: F Li (2015)
  www.caister.com/thermophile
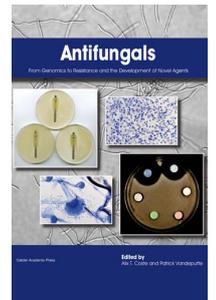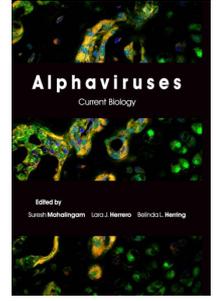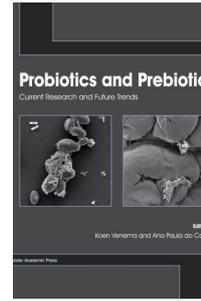
- **Flow Cytometry in Microbiology: Technology and Applications**
  Edited by: MG Wilkinson (2015)
  www.caister.com/flow

- **Probiotics and Prebiotics: Current Research and Future Trends**
  Edited by: K Venema, AP Carmo (2015)
  www.caister.com/probiotics

- **Epigenetics: Current Research and Emerging Trends**
  Edited by: BP Chadwick (2015)
  www.caister.com/epigenetics2015

- *Corynebacterium glutamicum*: **From Systems Biology to Biotechnological Applications**
  Edited by: A Burkovski (2015)
  www.caister.com/cory2

- **Advanced Vaccine Research Methods for the Decade of Vaccines**
  Edited by: F Bagnoli, R Rappuoli (2015)
  www.caister.com/vaccines

- **Antifungals: From Genomics to Resistance and the Development of Novel Agents**
  Edited by: AT Coste, P Vandeputte (2015)
  www.caister.com/antifungals

- **Bacteria-Plant Interactions: Advanced Research and Future Trends**
  Edited by: J Murillo, BA Vinatzer, RW Jackson, et al. (2015)
  www.caister.com/bacteria-plant

- *Aeromonas*
  Edited by: J Graf (2015)
  www.caister.com/aeromonas

- **Antibiotics: Current Innovations and Future Trends**
  Edited by: S Sánchez, AL Demain (2015)
  www.caister.com/antibiotics

- *Leishmania*: **Current Biology and Control**
  Edited by: S Adak, R Datta (2015)
  www.caister.com/leish2

- *Acanthamoeba*: **Biology and Pathogenesis (2nd edition)**
  Author: NA Khan (2015)
  www.caister.com/acanthamoeba2

- **Microarrays: Current Technology, Innovations and Applications**
  Edited by: Z He (2014)
  www.caister.com/microarrays2

- **Metagenomics of the Microbial Nitrogen Cycle: Theory, Methods and Applications**
  Edited by: D Marco (2014)
  www.caister.com/n2
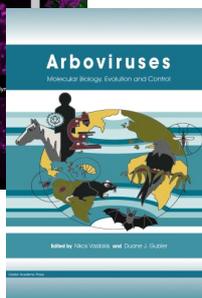
Order from caister.com/order

animals. Salmonella and yersinia have also been linked to UC (Sartor *et al.,* 1996), while experiments on transgenic rats showed that colonic inflammation was related to numbers of bacteroides in the gut (Rath *et al.,* 1996; 1999).

Certain strains of *E. coli* isolated from the colitic bowel have increased adhesive properties (Chadwick, 1991), although this may be an adaptation to the disease state in the host. *Escherichia coli* has been implicated in UC in a number of studies, and isolates from faeces and rectal biopsies in UC patients have been reported to have greater adherent abilities than those obtained from healthy people or individuals with infectious diarrhoea (Burke and Axon, 1988). However, other investigators have not found this to be the case (Hartley *et al.,* 1993; Schultsz *et al.,* 1997), while some workers observed that *E. coli* occurred in only a small number of tissue samples taken from UC patients (Walmsey *et al.,* 1998).

Bacterial L-forms, or cell wall deficient organisms have been found in both UC and CD . These organisms lack cell wall antigens, and so are less visible to host immune surveillance and can survive intracellularly. L-forms, mainly *Enterococcus faecalis* and *E. coli*, were detected in 42% of patients with UC (n=121) and in 34% of CD patients (n=71), but in only 1% of healthy controls (n=140). It is not clear, however, whether these cell forms actually play a role in the aetiology of UC, or whether they are organisms adapted to the different nutritional and environmental conditions that occur in the inflamed bowel. However, if L-forms are aetiologic agents in UC, it would partly explain why pathogenic bacteria have not been visualised in, or isolated from diseased mucosae in IBD patients.

In general, there is no real evidence for a specific transmissible agent in UC in humans, as indicated by the work of Victor *et al.,* (1950), who injected cell-free filtrates of stool and rectal mucosa from IBD patients into the monkey colon, and failed to induce inflammation. Furthermore, the majority of bacteria, which have been implicated in various studies, are not found in all patients with the disease. Nevertheless, there is a good case for bacteria growing on the gut wall playing a major role in IBD, either as pathogenic organisms proliferating on the epithelial surface and invading the underlying mucosa, or alternatively, by non-pathogenic commensals occupying adhesion sites on the mucosa and preventing colonisation by harmful species. This was indicated to occur when non-pathogenic *Escherichia coli* were used to displace resident strains in UC patients (Rembacken *et al.,* 1999).

Dissimilatory Sulphate-Reducing Bacteria (SRB) and UC

SRB are normal inhabitants of the human large intestine. However, an intracellular Gram-negative bacterium, known as ileal symbiont intracellularis (ISI) related to desulfovibrios, has been associated with bloody diarrhoea, weight loss and anorexia in ferrets, hamsters and pigs (Gebhardt *et al.,* 1993; Fox *et al.,* 1994). This condition is infectious, and histology shows epithelial hyperplasia, goblet cell depletion, crypt abscesses and inflammatory cell infiltrates. Cultivation of these bacteria has been unsuccessful, but when grown in tissue culture and inoculated into hamsters, they induce characteristic inflammatory changes. However, ISI does not seem to play a similar role in human IBD. When rectal biopsies from 19 UC patients were studied using an immunofluorescent assay, with mouse monoclonal antibody IG4 targeted to ISI (Pitcher, 1996), none of these bacteria were observed, although fluorescent curved bacilli were demonstrated to be present in the apical cytoplasm of biopsies from a pig with porcine proliferative enteropathy. These experiments also established that there was no reactivity against eight SRB isolates cultured from UC patient faeces.

In a study of 87 healthy subjects in the UK, SRB were isolated from faeces from 66 individuals (76%), with the predominant species being members of the genus *Desulfovibrio* (Gibson *et al.,* 1991, 1993). Growth of mucosal-associated SRB was detected in 92% of UC samples as compared to 52% in controls (Zinkevich and Beech, 2000). In these studies, PCR analysis further indicated that SRB were ubiquitous, and were present in all of these biopsies studied. However, a recent investigation, in France, involving 151 subjects (Loubinoux *et al.,* 2002) found a lower prevalence of SRB in faeces in healthy subjects (12%), while the incidence of SRB (desulfovibrios) was significantly higher in IBD (UC and CD) patients (55%).

Gibson *et al.,* (1991) observed that although SRB occurred in lower numbers in UC faeces, compared to stools from healthy subjects, their metabolic activities, as assessed by specific rates of sulphate reduction, were considerably higher than in the controls. It was also shown that SRB isolated from UC patients were particularly adapted to grow under low sulphate concentrations, at high specific growth rates, which was related to ecological selection by environmental conditions in the colitic bowel.

The significance of SRB in the large intestine is that sulphate is used by these bacteria as a terminal electron acceptor in metabolism, where it is ultimately reduced to sulphide, a strong cellular toxin (Pitcher *et al.,* 1998). Hydrogen sulphide has been shown to inhibit butyrate oxidation in colonocytes (Roediger *et al.,* 1993), evoke cellular hyperproliferation in the colonic mucosa, and to induce colonocyte metabolic abnormalities similar to those that occur in UC (Christl *et al.,* 1996). In rats, perfusion of the colon with physiological concentrations of sulphide leads to superficial ulceration of the bowel (Aslam *et al.,* 1992), while its immunomodulatory effects inhibit the abilities of polymorphonuclear leucocytes to phagocytose and kill bacteria, which may facilitate their translocation in the gut (Gardiner *et al.*, 1995). Pitcher *et al.,* (2000) showed that untreated UC patients had significantly higher concentrations of sulphide in faeces, when compared to healthy controls, and that 5-ASA, the main drug used to treat UC, strongly inhibited sulphide production *in vivo* and *in vitro*. However, despite the undoubted toxicological effects of sulphide in *in vitro* model systems, and in animal studies, evidence for this bacterial metabolite acting as a specific cellular toxin in UC remains circumstantial (Cummings and Macfarlane, 1999).

A number of investigations have now linked SRB to UC, but whether these bacteria are aetiologic agents, or are simply taking advantage of changes in the gut

ecosystem resulting from mucosal inflammation, tissue destruction and diarrhoea is unclear. Sulphide is produced by amino acid fermenting anaerobes in the large bowel, as well as by SRB. Thus, even if this metabolite is an important mucosal toxin in UC, the presence or absence of SRB, as well as their metabolic propensities, does not necessarily explain the prevalence or severity of the disease.

Antibody Responses to the Normal Microflora in UC Patients

Compared to healthy people, UC patients have greatly increased levels of mucosal IgG directed against members of the normal colonic microbiota, which have been suggested to contribute to relapse (Macpherson *et al.,* 1996). These workers tested a range of bacterial proteins prepared from *Bacteroides fragilis*, *E. coli*, *Clostridium perfringens*, *Enterobacter faecalis*, *Staphylococcus epidermidis*, *Haemophilus influenzae* and *Klebsiella aerogenes* against mucosal IgG obtained from intestinal washings in UC and CD patients. Binding was observed to occur with *B. fragilis*, *C. perfringens* and *E. coli*. In contrast, there was little binding of mucosal IgG to the non-intestinal species, although serum IgG titres were high to these organisms.

Monteiro *et al.,* (1971) found increased antibody production against strictly anaerobic species, and UC patients are known to have increased antibody titres to bacteroides (Tvede *et al.,* 1983), particularly *B. vulgatus* (Bamba *et al.,* 1995), where the response was to a low molecular mass protein. Similarly, a recent study has suggested that *Bacteroides ovatus* was one of the principal colonic microorganisms eliciting the systemic antibody response (IgG and IgA) in UC and CD patients (Saitoh *et al.,* 2002). This was attributed to a 19.5 kDA protein, though its role in disease aetiology was unclear. Other evidence linking bacterial proteins to the host immune response in UC indicated that *E. coli* outer membrane protein ompC, and a 100 kDA protein of *Bacteroides caccae* were immunoreactive to a pANCA monoclonal antibody (Cohavy *et al.,* 2000), which is produced in response to a neutrophil protein in the majority of UC patients (Satsangi *et al.,* 1998).

Matsuda *et al.,* (2000) reported high serum antibody responses against *Bacteroides vulgatus*, *B. fragilis* and *Clostridium ramosum* in UC patients. With respect to *B. vulgatus*, 54% of UC patients had IgG antibodies active against a 26 kDa outer membrane protein, compared to 9% in healthy subjects. These antibodies were suggested to play a role in UC aetiology. This investigation also showed that 29% of UC patients produced IgG antibodies against a 50 kDa *E. coli* protein, against only 6% of controls.

Mucosal bacterial populations
Since bacteria growing on the mucosal surface in the large intestine exist in close juxtaposition to host tissues, it might intuitively be expected that these organisms interact to a greater extent with the host immune and neuroendocrine systems than their lumenal counterparts. It is therefore surprising that few investigations have been made on the bacteria that inhabit the colonic mucosa surface compared to those present in the gut lumen. There are several reasons for this: firstly, faeces and material from the lumen of the bowel are readily available for study, while in healthy individuals, there are practical and ethical problems in obtaining fresh mucosal biopsy tissue.

Nevertheless, there is now sufficient evidence to suggest that bacterial populations colonising the large bowel mucosa are different in composition to those in the gut lumen (Macfarlane *et al.,* 1999, 2000). Using colonic biopsies taken from healthy subjects, Hartley *et al.,* (1979) found that while bacteria adhered directly to the bowel wall, they were present in higher numbers in the mucus layer. These studies indicated that one *E. coli* strain predominated in each tissue sample, and that this strain occurred throughout the length of the gut. Croucher *et al.,* (1983) studied colonic tissue from different regions of the bowel in four sudden death victims, none of whom had UC, and noted the existence of distinct bacterial communities in each individual. It was also reported that anaerobe : aerobe ratios were much lower than in the gut lumen, at about $10^4 : 1$. However, bacteroides, fusobacteria and bifidobacteria predominated. Microscopic analysis suggested that the majority of organisms occurred under the top surface of the mucus layer.

Poxton *et al.,* (1997) reported that viable counts on mucosal tissue taken during colonoscopy were comparable in the rectum and proximal colon, and that numbers of anaerobic species were 10 to 100-fold higher than those of aerobes and facultative anaerobes. Six patients with UC were studied, together with six individuals with non-inflammatory bowel conditions. Few consistent or significant differences in mucosal bacterial populations were evident in the two groups, however, *Bacteroides thetaiotaomicron* seemed to have a higher prevalence in the UC patients. These studies further showed that bacteroides predominated on mucosal surfaces, accounting for up to 69% of total anaerobe counts, with *B. vulgatus* being the dominant species.

Comparison of bacterial communities in biopsies from normal and inflamed colonic mucosa in patients with acute UC showed significant reductions in lactobacilli in the IBD group, as well as lesser numbers of bifidobacteria, while *B. thetaiotaomicron* increased in frequency (Pathmakanthan *et al.,* 1999). The authors concluded that reduced numbers of mucosal lactobacilli in UC enabled the bacteroides, as well as other putatively pathogenic species to colonise the bowel wall.

More recently, Matsuda *et al.,* (2000) reported that counts of both aerobic and anaerobic bacteria increased on the rectal mucosa of UC patients, and that *Bacteroides vulgatus* predominated. These experiments indicated that the frequency of isolation of bacteroides, peptostreptococci, *Clostridium ramosum* and *Bifidobacterium breve* was higher in UC, and that fusobacteria were only present in healthy controls.

Other studies, using fluorescently labelled 16S rRNA oligonucleotide probes, have indicated that the mucus layer in rectal biopsies is more heavily colonised by bacteria in IBD patients, including those with UC (Schultsz *et al.,* 1999). These investigations found no bacteria in 71% of the control subjects and 32% of the IBD patients, which is surprising,

since, as related above, culturing studies consistently show the rectal mucosa to harbour large numbers of bacteria in both health and disease.

Antibiotics

Animal Models

Since Marcus and Watt described the first reliable animal model of colitis, feeding carrageenan to guinea pigs, there have been a number of descriptions of colitis induced in other conventional species (rat, mouse, hamster, rabbit and monkeys, including the Cotton Top Tamarin), at least twelve knockout or transgenic animals and using a dozen or more different agents, such as dextran sulphate, amylopectin sulphate, TNBS (trinitrobenzene sulphonic acid) etc (Watt and Marcus, 1973; Marcus and Watt, 1969; Elson *et al.*, 1995; Fiocchi, 1998). It is almost invariable in these widely differing models that the presence of a microflora is needed to induce colitis, and as Table 1 shows, that the severity of the inflammatory process can be ameliorated by certain antibiotics. Can we learn anything about the role of bacteria in UC from these models?

Firstly, not all antibiotic regimes prevent or improve colitis in animals. Eleven antibiotics are used in the papers described in Table 1, of which metronidazole, first reported by Bartlett's group (Onderdonk *et al.,* 1977) is most consistently beneficial. Metronidazole is an absorbed antibiotic and antiprotozoal drug active against anaerobic microorganisms, and it is one of the most widely used antibiotics for anaerobic infections in current clinical practice. The lack of effect of metronidazole in the study by Videla *et al.*, (1994) may be because it was given by enema, although enema and suppository are usually successful routes of delivery when treating human infections. Perhaps antibiotics just do not work with the TNBS model. Moreover, the timing of drug administration may be important, since in most of the studies in Table 1, the benefit of metronidazole, or other antibiotics, is seen only when the drug is given before colitis is induced.

None of the other antibiotics used in these animal studies have such good effects against intestinal anaerobes. Several, notably gentamycin, tobramycin, neomycin and streptomycin have been chosen because of their activity against Gram-negative facultative anaerobes, especially *E. coli*. This gut commensal and common pathogen is readily cultured and was one of the first bacteria to be specifically implicated in UC (Monteiro *et al.*, 1971; Van der Waaij *et al.*, 1974; Burke and Axon, 1988). However, these aminoglycoside antibiotics are singularly lacking in effect in preventing or curing animal colitis, and as mentioned earlier, the role of *E. coli* as a primary pathogen in UC has never been established. The combination of trimethoprim-sulphamethoxazole, used only in specific human infections such as *Pneumocystis carinii* and toxoplasmosis, because of its toxicity, is also active against enterobacteria, and it produced modest benefit in reducing disease severity in two studies, although it by no means prevented ulceration in the colon (Van der Waaij *et al.*, 1974; Onderdonk and Bartlett, 1979).

Vancomycin, a glycopeptide antibacterial agent that is not absorbed from the gut, is active particularly against Gram-positive cocci and some anaerobes. Although there are increasing reports of resistant enterococci in humans, it has been a popular choice of antibiotic in animal studies. Used alone it is not effective, nor in combination with tobramycin, but it was consistently beneficial in three different models, when combined with impinemem. In the study by Rath *et al.,* (2001), this combination was best at both preventing and treating colitis in both HLA-B27 transgenic rats and dextran sulphate treated mice. Impinemem given alone is without benefit (Videla *et al.*, 1994). Impinemem is another non-absorbable antibiotic active against Gram-positive cocci, like vancomycin, but while it is also active against Gram-negative bacilli and some anaerobes, this drug is not currently used in human medicine.

Clindamycin is similarly active against Gram-positive cocci, but also against many anaerobes. It has very limited and specific uses in human infection because it is the antibiotic most likely to cause antibiotic associated diarrhoea. Thus it might be predicted that clindamycin has a profound effect on the microflora, and in the only study reported of its use in animals, it was found to be very effective in preventing ulceration in guinea pigs (Onderdonk and Bartlett, 1979).

Penicillins are virtually never used in animal models, but one study (Videla *et al.*, 1994) has reported the use of amoxicillin with clavulanic acid by rectal enema in the rat TNBS model. This treatment was beneficial both acutely (7 days) and in the longer term (21 days). Amoxicillin is a broad spectrum penicillin that is well absorbed, diffuses readily through tissues and is active against both Gram-negative and Gram-positive species. It is inactivated by penicillinases, but when combined with the ß-lactamase inhibitor clavulanic acid, the combination is active against ß-lactamase producing species including *E. coli*, many bacteroides and klebsiella.

A currently popular antibiotic for gastrointestinal infection is ciprofloxacin, which is employed successfully in CD (Vermeire and Rutgeerts, 2001). This drug has been used in three animal models of colitis, and was clearly beneficial, especially in preventing disease (Madsen *et al.*, 2000; Hans *et al.*, 2000; Rath *et al.*, 2001), but it was less effective in managing established colitis. Combination with metronidazole was also beneficial. Ciprofloxacin is a fluoroquinolone that is well absorbed and is active against Gram-negative facultative anaerobes and microaerophiles, such as salmonella, shigella and campylobacter, and it also has modest activity against Gram-positive organisms such as *Enterococcus faecalis*.

Is there any common theme or lesson to be learnt from these studies? To be effective an antibiotic for animal colitis should have activity against gut anaerobes. Those antibiotics that specifically target Gram-negative facultative species such as *E. coli* are not successful in IBD. Some combination of antibiotics, e.g. vancomycin and impinemem, seem better than the drugs given separately. This may simply reflect better combined anaerobe activity. Thus the most consistently effective drugs are metronidazole, clindamycin and ciprofloxacin, if given before onset of colitis, and the vancomycin/impinemem combination. Apart from their activity against anaerobes,

Table 1. Antibiotic use in animal models of colitis

Species	Model	Antibiotic	Results	Source
Guinea pig	Carrageenan 2 or 5%	Trimethoprim + sulphamethoxazole	Reduced severity of ulceration	Van der Waaij et al., 1974
Guinea pig	Carrageenan 5%	Gentamycin Trimethoprim + sulphamethoxazole Vancomycin Clindamycin Metronidazole Germ free	No effect on ulceration but reduced mortality Prevented ulceration Reduced ulceration No ulceration	Onderdonk and Bartlett, 1979) Onderdonk et al, 1977) Onderdonk et al, 1978)
Rat	TNBS	Amoxicillin + clavulanic acid Tobramycin Impinenem + vancomycin Metronidazole Tobramycin + vancomycin	Improved at 7 and 21 days No benefit Improved at 7 and 21 days No benefit No benefit	Videla et al, 1994
Mouse	IL 10 knockout	Germ free SPF or populated with assorted species	Did not develop colitis All developed colitis	Sellon et al, 1998
Rat	Caecal self-filling blind loop in SPF HLA-B27 transgenics	Metronidazole	Attenuated inflammation and eliminated bacteroides	Rath et al, 1999
Mice	BALB/c with DSS 5%	Ciprofloxacin + metronidazole I-P	Prevented colitis but no benefit if already established	Hans et al, 2000
Mouse	IL-10 deficient	Ciprofloxacin Metronidazole + neomycin	Both regimes prevented colitis when given early and improved established colitis	Madsen et al, 2000
Rat	HLA-B27 transgenic	Ciprofloxacin Metronidazole Vancomycin + impinenen	Metronidazole prevented colitis in both models but no benefit as treatment Ciprofloxacin partly beneficial in HLA-B27 prevention only	Rath et al, 2001
Mouse	DSS 5%	Metronidazole Vancomycin + impinenem	Vancomycin + impinenem best at treatment and prevention in both models	

Abbreviations
TNBS   Trinitrobenzene sulfonic acid
SPF   Specific pathogen free
DSS   Dextran sodium sulphate
I-P   Intraperitoneal

these antibiotics also target Gram-positive bacteria such as enterococci. Are these findings reflected in human studies of antibiotic use in UC?

Human Studies in UC
More than a dozen randomised controlled trials (RCT) of antibiotic use in UC have been published in recent years. Six antibiotics have been used, given orally in all but three studies, and singly, apart from one study (Table 2). Metronidazole has been the subject of four trials and given its value in animal models of UC, it might be hoped that it would be equally successful in humans. However, in only one of the four reported studies was metronidazole shown to be beneficial. Gilat *et al.*, (1987, 1989) first treated 42 UC patients in an acute attack for 28 days with 1.35 grams metronidazole per day by mouth, and found no benefit over treatment with sulfasalazine in an RCT. Only 26% improved with antibiotic, versus 68% with sulfasalazine. They then followed the patients for one year, giving 0.6 grams metronidazole, or 2 grams sulfasalazine per day. Metronidazole was found to be "slightly more effective" after 12 months of treatment. No side effects were reported, which given the propensity for metronidazole to cause peripheral neuropathy in long term use, was reassuring. In addition to these studies by Gilat and co-workers, two other investigations with metronidazole have been reported, one of which was combined with tobramycin. Neither showed benefit. However, three of the four reported studies were short term, 5-28 days, treating acute attacks of colitis. In two investigations (Chapman *et al.*, 1986; Mantzaris *et al.*, 1994), the antibiotics were given intravenously. The use of intravenous antibiotic therapy in acute severe UC was first reported by Truelove and Jewell (1974), when tetracycline was incorporated into a bag containing glucose/saline, a parenteral feed, steroids and vitamins. Thirty six out of 49 patients improved rapidly, but this was not an RCT, and no conclusions regarding the efficacy of tetracycline can be drawn from it. As far as metronidazole is concerned, it would not appear to be of benefit in acute colitis, but the drug is worth testing in more long term studies.

Tobramycin appears to be more successful in acute attacks. Axon's group (Burke *et al.*, 1990) found 74% of patients in remission at 28 days, following a 7 day course of oral antibiotic, compared with 43% on placebo. The same group found no benefit for tobramycin at 12 and 24 months in 81 patients (Lobo *et al.*, 1993). Overall therefore, tobramycin chosen principally for its effect against Gram-negative species such as *E. coli*, showed no consistent benefit.

The first RCT of antibiotics to treat UC was also done by the Leeds group (Dickinson *et al.*, 1985) using vancomycin (2 grams given orally per day), which was chosen because of its benefits in antibiotic associated diarrhoea. In 33 cases of acute UC, there was no clear benefit although fewer came to surgery in the antibiotic group (2/18) compared to the placebo group (7/15).

Whilst the objective of using antibiotics in UC is to change the microflora beneficially, it is surprising that very few studies include reports of any bacteriology done on the patients. One of two studies in which ciprofloxacin has been used (Turunen *et al.*, 1998), included semi-quantitative estimates of mucosal bacteria from endoscopic biopsies. They reported no enteric pathogens at the start of the study, and the disappearance of Gram-negative facultative anaerobes in the antibiotic group. After six months of ciprofloxacin treatment, the failure rate was 21% in the treated group, and 44% with the placebo (p = 0.02). However, endoscopic and histological changes were no different between these groups at six months, and there was no clinical benefit at one year. Serum IgG, IgM and IgA antibodies to *E. coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* were all higher at entry to the study in the UC patients. During treatment with ciprofloxacin, IgG antibodies to *E. coli* fell in the treated group and these bacteria disappeared from stools (Turunen *et al.*, 1999).

The other two investigations with ciprofloxacin (Mantzaris *et al.*, 1997, 2001) were in acute colitis of varying degree of severity, and they showed no benefit for the antibiotic.

One study with amoxicillin/clavulanic acid has been published (Casellas *et al.*, 1998). The drug was given orally for 5 days to patients who had an acute attack without apparent benefit, except in the release of inflammatory mediators, quantitated by mucosal release of eicosanoids using rectal dialysis. A report published only in abstract some years ago (Danzi, 1989) suggests trimethoprim-sulphamethoxazole is of benefit in severe total UC, possibly because of its anti-folate (immunosuppressive) effect.

Rifaximin is a new generation antibiotic targeted at the gut, that is derived from rifamycin. It is non-absorbable (Gionchetti *et al.*, 1997) and has a broad spectrum of activity against both Gram-positive and Gram-negative bacteria, as well as colonic anaerobes. Because of its activity against pathogens such as *E. coli*, salmonella and shigella, it is being used for traveller's diarrhoea and appears to be as good as ciprofloxacin (DuPont *et al.*, 1998; Steffen, 2001). Rifaximin is poorly absorbed, even in UC and pouchitis patients with inflamed mucosae (Rizzello *et al.*, 1998; Gionchetti *et al.*, 1999), and high concentrations can be found in stools (Jiang *et al.*, 2000). This drug has been used successfully to treat pouchitis, in combination with ciprofloxacin (Gionchetti *et al.*, 1999), and there are now two reports of its use in UC (Rizzello *et al.*, 1997; Lukas *et al.*, 2002). In an open label study, 31 patients with mild to moderate, predominately left sided UC, took 400 mg twice daily for 10 days. At 28 days, the clinical activity index and sigmoidoscopy scores were significantly better, and only two patients were worse (Lukas *et al.*, 2002). In the Rizzello *et al.*, (1997) RCT, the same dose was used in moderate to severely affected, steroid resistant UC patients. Sixty four percent of treated patients were substantially better versus 42% of the placebo group, and significant improvement was seen in stool frequency, rectal bleeding and sigmoidoscopy scores. In a separate study using this same group of patients, 1.8 grams of rifaximin were given daily for three treatment periods of 10 days, to 12 UC patients and the effects on the faecal microflora were characterised (Brigidi *et al.*, 2002). The antibiotics suppressed lactobacilli, bifidobacteria, bacteroides and *Clostridium perfringens*, but the numbers returned to their initial values during the 25 day washout period. At the

Table 2. Clinical trials of antibiotic use in ulcerative colitis

Antibiotic	Dose/day	Route	Duration	N	Study design Results	Source
Metronidazole	1.5 g	I-V	5 days	39	RCT severe UC No benefit	Chapman et al., 1986
Metronidazole	1.35 g	Oral	28 days	42	RCT v. salazopyrine No benefit	Gilat et al., 1987
					4.5 g. Acute attack	
Metronidazole	0.6 g	Oral	12 months	33	RCT v. salazopyrine 2 g. Maintenance of remission Benefit at 12 months, also longer remission	Gilat et al., 1989
Tobramycin and metronidazole	12 mg/kg	I-V	10 days	39	RCT Acute severe UC No benefit	Mantzaris et al., 1994
	1.5 g					
Tobramycin		Oral	7 days	84	RCT Acute UC At 28 days tobramycin better Burke et al., 1990	
Tobramycin		Oral	7 days	81	RCT Long-term follow up No benefit at 12 or 24 months	Lobo et al., 1993
Vancomycin	2 g	Oral	7 days	33	RCT Acute UC No clear benefit but less surgery Dickinson et al., 1985	
Ciprofloxacin	1–1.5 g	Oral	6 months	83	RCT Turunen et al., 1998	
					Benefit at 6 months, but not at 12	
Ciprofloxacin	800 mg	I-V	10 days	39	RCT Acute severe UC No benefit	Mantzaris et al., 2001
Ciprofloxacin	500 mg	Oral	14 days	70	RCT Mild or moderate acute UC No benefit	Mantzaris et al., 1997
Amoxicillin-clavulanic acid	2.25 g	Oral	5 days	30	RCT Acute attack Release of inflammatory mediators reduced	Casellas et al., 1998
Rifamixin	800 mg	Oral	10 days	28	RCT Moderate to severe steroid resistant UC Substantial clinical and endoscopic benefit	Rizzello et al., 1997
Rifamixin	800 mg	Oral	10 days	31	Open label Active UC Effective in patients with mild to moderate left-sided UC	Lukas et al., 2002

Abbreviations
IV   Intravenous
RCT   Randomised controlled trial
UC   Ulcerative colitis

Table 3. Problems associated with antibiotic use in ulcerative colitis. Modified from Cummings and Macfarlane (2001)

Bacteria involved not known, therefore no antibiotic sensitivities

Bacteria may be part of a biofilm consortium on the mucosal surface

Therapeutic levels of antibiotics may not be reached at target site

Resistance developing, especially with long term use

Effect of antibiotic on microflora and barrier resistance to pathogens

Timing of use. Animal studies suggest antibiotics need to be given before onset of inflammation

Systemic side-effects

present time, rifamixin has the best record for treating UC, but long term studies are clearly needed to fully evaluate its worth.

Antibiotics are not used routinely in clinical practice for either the acute attack or maintenance of remission in UC. Evidence for their effectiveness in both animal studies and human RCT's is less than compelling. Why should this be the case when so much evidence points to the gut microflora playing an essential role as a causative agents in the inflammatory process in UC, and when antibiotics are used so successfully in CD and pouchitis?

Firstly, we do not as yet know precisely what bacteria, or groups of bacteria are principally involved in UC (see Table 3). Over the years many different species have been implicated, but none have stood the test of time (Cummings and Macfarlane, 2001). Studies of faecal bacteria in UC have been largely unhelpful, possibly because of the many hours of work required to characterise these microfloras, in even a simple way. The investigator is faced with finding a needle in a haystack, although new techniques for identifying bacteria by chemotaxonomy and molecular typing may be more productive. What physician would treat an infection, especially long term, without knowing the causative agent and its antibiotic sensitivities?

Another important reason why antibiotics do not apparently work in UC is that the causative bacteria may well be present on the epithelial surface, as part of a biofilm community. In this state they are more resistant to antibiotics (Anwar *et al.,* 1990). Furthermore, we do not know if therapeutic levels of antibiotics reach the mucosal interface with biofilm bacteria. Many antibiotics used, such as tobramycin, rifamixin and vancomycin are not absorbed. Conversely, well absorbed antibiotics such as metronidazole achieve only very low concentrations in lumenal contents in the large bowel.

Other important problems concern the development of bacterial antibiotic resistance, with prolonged use, and systemic side effects which are more likely long-term. Timing is another important factor to take into consideration. Animal studies suggest that most benefit is achieved if antibiotics are given before the onset of colitis. This is clearly not possible in UC, but it may be an argument for maintenance antibiotic therapy in patients who are in remission.

Finally, most current antibiotics are active against several bacterial species, and their capacity to breakdown the normal barrier function of the microflora in the gut is well known. Antibiotics, unless very specifically targeted, could just get rid of one problem species, while facilitating the introduction of others. A more effective long-term treatment may lie in the use of probiotics.

Probiotics in UC and Pouchitis

Surprisingly few studies have been reported on the effects of probiotics in UC. However, a probiotic mixture comprising three bifidobacteria, four lactobacilli and a streptococcus has been used in an uncontrolled investigation with 20 UC patients in remission, over a 12 month period (Venturi *et al.,* 1999). Large doses of probiotic were involved (six grams were given per day, with bacterial counts corresponding to 5×10^{11} per gram). Microbiological analysis of faeces showed high numbers of probiotic bacteria during feeding, although total anaerobic and aerobic counts, together with clostridia, bacteroides, enterobacteria were not significantly affected. Fifteen of 20 patients were maintained in remission during probiotic feeding, while four relapsed. This probiotic was subsequently used in an RCT where 17 out of 20 pouchitis patients remained in remission for nine months while taking the probiotic, all of whom relapsed within a few months after the treatment was stopped (Campieri and Gionchetti, 1999).

Lactobacilli have been reported to prevent colitis in IL-10 deficient mice (Madsen *et al.,* 1999) and to reduce colitis in rats induced with either acetic acid (Fabia *et al.,* 1993a) or methotrexate (Mao *et al.,* 1996). In studies with *Lactobacillus plantarum*, the bacterium was shown to stabilise the gut mucosal barrier in IL-10 deficient mice with colitis (Kennedy *et al.,* 2000), whereas when *Lactobacillus plantarum* 299V was used to prevent and treat spontaneous colitis in the IL-10 mouse model of colitis (Schultz *et al.,* 2002), the probiotic reduced mucosal IgG, interferon-γ and IL-12, and attenuated established inflammatory processes.

Reduced numbers of lactobacilli in colonic biopsies were found in pouchitis patients (Fabia *et al.,* 1993b), while pouchitis patients were reported to have low numbers of lactobacilli and bifidobacteria in gut contents by Ruseler van Embden *et al.,* (1994), suggesting that they may a protective role in UC.

In two RCT, a non-pathogenic *E. coli* was compared with mesalazine in patients either in remission , over 12 weeks, or after relapse , over 12 months. Both studies found the probiotic equally as effective as mesalazine in preventing relapse.

To date, probiotic studies in UC have provided little insight into the microbiological and immunological mechanisms involved in inflammatory bowel diseases. While probiotics and possibly prebiotics do show some promise as therapies in IBD, until more is known about the mucosa-associated microflora and the mechanisms of inflammation, their employment will remain largely empirical.

References

Anwar, H., Dasgupta, M.K., and Costerton, J.W. 1990. Testing the susceptibility of bacteria in biofilms to antibacterial agents. Antimicrob. Ag. Chemother. 34: 2043-2046.

Aslam, M., Batten, J.J., Florin, T.H.J., Sidebotham, R.L., and Baron, J.H. 1992. Hydrogen sulphide induced damage to the mucosal barrier in the rat. Gut. 33: S69.

Bamba, T., Matsuda, H., Endo, M., and Y. Fujiyama, Y. 1995. The pathogenic role of *Bacteroides vulgatus* in patients with ulcerative colitis. J. Gastroenterol. 30 (Suppl. 8): 45-47.

Belsheim, M., Darwisk, R., and M. Watson, M. 1983. Bacterial L-form isolation from inflammatory bowel disease patients. Gastroenterology. 85: 364-369.

Brigidi, P., Swennen, E., Rizzello, F., Bozzolasco, M. and Matteuzzi, D. 2002. Effects of rifaximin administration on the intestinal microbiotia in patients with ulcerative colitis. J. Chemotherapy. 14: 290-295.

Burke, D., and Axon, A. 1988. Adhesive *E. coli* in inflammatory bowel disease and infective diarrhoea. BMJ. 297: 102-104.

Burke, D.A., Axon, A.T.R., Clayden, S.A., Dixon, M.F., Johnston, D. and Lacey, R.W. 1990. The efficacy of tobramycin in the treatment of ulcerative colitis. Aliment. Pharmacol. Therapeut. 4: 123-129.

Campieri, M. and Gionchetti, P. 2001. Bacteria as the cause of ulcerative colitis. Gut. 48: 132-135.

Campieri, M., and Gionchetti, P. 1999. Probiotics in inflammatory bowel disease: New insight to pathogenesis or a possible therapeutic alternative? Gastroenterology. 116: 1246-1260.

Casellas, F., Borruel, N., Papo, M., Guarner, F., Antolin, M., Videla, S. and Malagelada, J.R. 1998. Antiinflammatory effects of enterically coated amoxicillin-clavulanic acid in active ulcerative colitis. Inflamm. Bowel Dis. 4: 1-5.

Chadwick, V.S. 1991. Etiology of chronic ulcerative colitis and Crohn's disease. In: The Large Intestine: Physiology, Pathophysiology and Disease. S.F. Phillips, J.H. Pemberton, and R.G. Shorter RG, ed. Raven Press Ltd., New York. p. 445-463.

Chapman, R.W., Selby, W.S. and Jewell, D.P. 1986. Controlled trial of intravenous metronidazole as an adjunct to corticosteroids in severe ulcerative colitis. Gut. 27: 1210-1212.

Christl, S., Eisner, H.D., Kasper, H., and Scheppach, W. 1996. Antagonistic effects of sulfide and butyrate on proliferation of colonic mucosa: a potential role for these agents in the pathogenesis of ulcerative colitis. Dig. Dis. Sci. 41: 2477-2481.

Cohavy, O., Bruckner, D., Gordon, L.K., Misra, R., Wei, B., Eggena, M.E., Targan, S.R., and Braun, J. 2000. Colonic bacteria express an ulcerative colitis pANCA-related protein epitope. Infect. Immun. 68: 1542-1548.

Cohen, M.B., and Giannella, R.A. 1991. Bacterial infections: pathophysiology, clinical features and treatment. In: The Large Intestine: Physiology, Pathophysiology and Disease. S.F. Phillips, J.H. Pemberton, and R.G. Shorter, ed. Raven Press Ltd., New York. p. 395-428.

Croucher, S.C., Houston, A.P., Bayliss, C.E., and Turner, R.J. 1983. Bacterial populations associated with different regions of the human colon wall. Appl. Environ. Microbiol. 45: 1025-1033.

Cummings, J.H., Banwell, J.G., Segal, I., Coleman, N., Englyst, H.N., and Macfarlane, G.T. 1990. The amount and composition of large bowel contents in man. Gastroenterology. 98: A408.

Cummings, J.H., Bingham, S.A.,. Heaton, K.W. and Eastwood, M.A. 1992. Fecal weight, colon cancer risk and dietary intake of non-starch polysaccharides (dietary fiber). Gastroenterology 103: 1783-1789.

Cummings, J.H. and Macfarlane, G.T. 1999. Are bacterial sulphur metabolites toxins in ulcerative colitis? In: *Helicobacter* meets inflammatory bowel disease. T. Shimoyama, A. Lee, A. Axon, D. K. Podolsky and C. O'Morain. ed. Axel Springer Japan Publishing Inc. p. 79-99.

Cummings, J.H. and Macfarlane, G.T. 2001. Is there a role for microorganisms? In: Challenges in inflammatory bowel disease. D.P. Jewell, B.F. Warren and N.J. Mortensen ed. Blackwell Science Ltd., Oxford. pp. 42-51.

Danzi, J.T. 1989. Trimethoprim-sulfamethoxazole therapy of inflammatory bowel disease. Gastroenterology. 96: A110.

Dickinson, R.J., O'Connor, H.J., Pinder, I., Hamilton, I., Johnston, D. and Axon, A.T.R. 1985. Double blind controlled trial of oral vancomycin in adjunctive treatment in acute exacerbation of idiopathic colitis. Gut. 26: 1380-1384.

DuPont, H.L., Ericsson, C.D., Mathewson, J.J., Palazzini, E., DuPont, M.W., Jiang, Z.D., Mosavi, A. and de la Cabada, F.J. 1998. Rifaximin: a nonabsorbed antimicrobial in the therapy of travelers' diarrhea. Digestion. 59: 708-714.

Elson, C.O., Sartor, R.B., Tennyson, G.S. and Riddell, RH. 1995. Experimental models of inflammatory bowel disease. Gastroenterology. 109: 1344-1367.

Eve. I.S. 1966. A review of the physiology of the gastro-intestinal tract in relation to radiation doses from radioactive materials. Health Phys. 12: 131-161.

Fabia, R, Ar'Rajab, A., Johansson, M.-L., Andersson, R., Willen, R., Jeppsson, B., Molin, G., and Bengmark, S. 1993b. Impairment of bacterial flora in human ulcerative colitis and experimental colitis in the rat. Digestion. 54: 248-255.

Fabia, R., Ar'Rajab, A., Johansson, M.-L., Willen, R., Molin, G., and Bengmark, S. 1993a. The effect of exogenous administration of *Lactobacillus reuteri* R2LC and oat fiber on acetic acid-induced colitis in the rat. Scand. J. Gastroenterol. 28: 155-162.

Farrell, R.J. and LaMont, J.T. 2002. Microbial factors in inflammatory bowel disease. Gastroenterol. Clin. North Am. 31: 41-62.

Farrell, R.J. and Peppercorn, M.A. 2002. Ulcerative colitis. Lancet. 359: 331-340.

Fiocchi, C. 1998. Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology. 115: 182-205.

Fox, J.G., Dewhirst, F.E., Fraser, G.J., Paster, B.J., Shames, B. and Murphy, J.C. 1994. Intracellular *Campylobacter*-like organism from ferrets and hamsters

with proliferative bowel disease is a *Desulfovibrio* sp. J. Clin. Microbiol. 32: 1229-1237.

Gardiner, K.R., Halliday, M.I., Barclay, G.R., Milne, L., Brown, D., and Stephens, S. 1995. Significance of systemic endotoxaemia in inflammatory bowel disease. Gut. 36: 897-901.

Gebhart, C.J., Barns, S.M., McOrist, S., Lin , G.-F., and Lawson, G.H.K. 1993. Ileal Symbiont Intracellularis, an obligate intracellular bacterium of porcine intestines showing a relationship to *Desulfovibrio* species. Int. J. Sys. Bacteriol. 43: 533-538.

Gibson, G.R., Cummings, J.H., and Macfarlane, G.T. 1991. Growth and activities of sulphate-reducing bacteria in gut contents of healthy subjects and patients with ulcerative colitis. FEMS Microbiol. Ecol. 86: 103-112.

Gibson, G.R., Macfarlane, S., and Macfarlane, G.T. 1993. Metabolic interactions involving sulphate-reducing and methanogenic bacteria in the human large intestine. FEMS Microbiol. Ecol. 12: 117-125.

Gilat, T., Leichtman, G., Delpre, G., Eschar, J., Bar-Meir, S. and Fireman, Z. 1989. A comparison of metronidazole and sulfasalazine in the maintenance of remission in patients with ulcerative colitis. J. Clin. Gastroenterol. 11: 392-395.

Gilat, T., Suissa, A., Leichtman, G., Delpre, G., Pavlotzky, M., Grossman, A. and Fireman, Z. 1987. A comparative study of metronidazole and sulfasalazine in active, not severe, ulcerative colitis. An Israeli multicenter trial. J. Clin. Gastroenterol. 9: 415-417.

Gionchetti, P., Ferrieri, A., Venturi, A., Brignola, C., Peruzzo, S., Raule, E., Descombe, J.J., Picard, M. and Campieri, M. 1997. Rifaximin, a non-absorbable antibiotic in treatment of steroid-resistant ulcerative colitis: A double-blind placebo controlled trial. Gastroenterology. 112: A1072.

Gionchetti, P., Rizzello, F., Venturi, A., Ugolini, F., Rossi, M., Brigidi, P., Johansson, R., Ferrieri, A., Poggiologi, G. and Campieri, M. 1999. Antibiotic combination therapy in patients with chronic, treatment-resistant pouchitis. Aliment. Pharmacol. Ther. 13: 713-718.

Hans, A.U., Scholmerich, J., Gross, V. and Falk, W. 2000. The role of the resident intestinal flora in acute and chronic dextran sulfate sodium-induced colitis in mice. Eur. J. Gastroenterol. Hepatol. 12: 267-273.

Hartley, M.G., Hudson, M.J., Swarbrick, E.T, Gent, A.E., Hellier, M.D. and Grace, R.H. 1993. Adhesive and hydrophobic properties of *Escherichia coli* from the rectal mucosa of patients with ulcerative colitis. Gut. 34: 63-67.

Hartley, C.L., Neumann, C.S., and Richmond, M.H. 1979. Adhesion of commensal bacteria to the large intestine wall in humans. Infect. Immun. 23: 128-132.

Hooper, L.V., Wong, M.H., Thelin, A., Hansson, L., Falk, P.G. and Gordon, J.I. 2001. Molecular analysis of commensal host-microbial relationships in the intestine. Science. 291: 881-884.

Jiang, Z.D., Ke, S., Palazzini, E., Riopel, L. and Dupont, H. 2000. *In vitro* activity and fecal concentration of rifaximin after oral administration. Antimicrob. Ag. Chemother. 44: 2205-2206.

Kennedy, R.J., Hoper, M., Weir, H., Deodhar, K., Erwin, P., Sloan, J., Kirk, S.J. and Gardiner, K.R. 2000. Probiotic therapy stabilises the gut mucosal barrier in the IL-10 knockout model of mouse colitis. Br. J. Surg. 87: 669-670.

Kruis, W., Schutz, E., Fric, P., Fixa, B., Judmaier, G. and Stolte, M. 1999. Double-blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. Aliment. Pharmacol. Ther. 11: 853-858.

Linskens, R.K., Huijsdens, X.W., Savelkoul, P.H.M., Vandenbroucke-Grauls, C.M.J.E. and Meuwissen, S.G.M. 2001. The bacterial flora in inflammatory bowel disease: current insights in pathogenesis and the influence of antibiotics and probiotics. Scand. J. Gastroenterol. 36: 29-40.

Lobo, A.J., Burke, D.A., Sobala, G.M. and Axon, A.T.R. 1993. Oral tobramycin in ulcerative colitis: effect on maintenance of remission. Aliment. Pharmacol. Ther. 7: 155-158.

Loftus, E.V., Silverstein, M.D., Sandborn, W.J., Tremaine, W.J., Harmsen, W.S. and Zinsmeister, A.R. 2000. Ulcerative colitis in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and survival. Gut. 46: 336-343.

Loubinoux, J., Bronowicji, J.-P., Pereira, I.A.C., Moungenel, J.-L., and Faou, A.E. 2002. Sulfate-reducing bacteria in human feces and their association with inflammatory diseases. FEMS Microbiol. Ecol. 40: 107-112.

Lukas, M., Konecny, M. and Zboril. V. 2002. Rifaximin in patients with mild to moderate activity of ulcerative colitis: An open label study. Gastroenterology. 122: A434.

Macfarlane, G.T., and Gibson, G.R. 1995. Bacterial infections and diarrhea. In: Human Colonic Bacteria: Role in Nutrition, Physiology and Pathology. G.R. Gibson, and G.T. Macfarlane, ed. CRC Press, Boca Raton. p. 201-226.

Macfarlane, S., Cummings, J.H., and Macfarlane, G.T. 1999. Bacterial colonisation of surfaces in the large intestine. In: Colonic Microflora, Nutrition and Health. G.R. Gibson, and M. Roberfroid, ed. Chapman and Hall, London, p. 71-87.

Macfarlane, S., Hopkins, M.J., and Macfarlane, G.T. 2000. Bacterial growth and metabolism on surfaces in the large intestine. Microb. Ecol. Hlth. Dis. 2: 64-72.

Macpherson, A., Khoo, U.Y., Forgacs, I., Philpott-Howard, J., and Bjarnason, I. 1996. Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. Gut. 38: 365-375.

Madsen, K.L., Doyle, J.S., Jewell, L.D., Tavernini, M.M., and Fedorak, R.N. 1999. *Lactobacillus* species prevents colitis in interleukin 10 gene-deficient mice. Gastroenterology. 116: 1107-1114.

Madsen, K.L., Doyle, J.S., Tavernini, M.M., Jewell, L.D., Rennie, R.P. and Fedorak, R.N. 2000. Antibiotic therapy attenuates colitis in interleukin 10 gene-deficient mice. Gastroenterology. 118: 1094-1105.

Mantzaris, G.J., Archavlis, E., Christoforidis, P., Kourtessas, D., Amberiadis, P., Florakis, N., Petraki, K., Spiliadi, C. and Triantafyllou, G. 1997. A prospective randomized controlled trial of oral ciprofloxacin in acute ulcerative colitis. Am. J. Gastroenterol. 92: 454-456.

Mantzaris, G.J., Hatzis, A., Kontogiannis, P. and

Triadaphyllou, G. 1994. Intravenous tobramycin and metronidazole as an adjunct to corticosteroids in acute, severe ulcerative colitis. Am. J. Gastroenterol. 89: 43-46.

Mantzaris, G.J., Petraki, K., Archavlis, E., Amberiadis, P., Kourtessas, D., Christidou, A. and Triantafyllou, G. 2001. A prospective randomised controlled trial of intravenous ciprofloxacin as an adjunct to corticosteroids in acute, severe ulcerative colitis. Scand. J. Gastroenterol. 36: 971-974.

Mao, Y., Nobaek, S., Kasravi, B., Adawi, D., Stenram, U., Molin, G., and Jeppsson, B. 1996. The effects of *Lactobacillus* strains and oat fiber on methotrexate-induced enterocolitis in rats. Gastroenterology. 111: 334-344.

Marcus, R. and Watt, J. 1969. Seaweeds and ulcerative colitis in laboratory animals. Lancet. 2: 489-490.

Matsuda, H., Fujiyama, Y., Andoh, A., Ushijima, T., Kajinami, T., and Bamba, T. 2000. Characterization of antibody responses against rectal mucosa-associated bacterial flora in patients with ulcerative colitis. J. Gastroenterol. Hepatol. 15: 61-68.

Mayberry, J.F., Ballantyne, K.C., Hardcastle, J.D., Mangham, C. and Pye, G. 1989. Epidemiological study of asymptomatic inflammatory bowel disease: the identification of cases during a screening programme for colorectal cancer. Gut. 30: 481-483.

Metcalf, A.M., Phillips, S.F., Zinsmeister, A.R., MacCarty, R.L., Beart, R.W. and Wolff, B.G. 1987. Simplified assessment of segmental colonic transit. Gastroenterology. 92: 40-47.

Monteiro, E., Fossey, J., Shiner, M., Draser, B.S. and Allison, A.C. 1971. Antibacterial antibodies in rectal and colonic mucosa in ulcerative colitis. Lancet. 1: 249-251.

Montgomery, S.M., Morris, D.L., Thompson, N.P., Subhani, J., Pounder, R.E. and Wakefield, A.J. 1998. Prevalence of inflammatory bowel disease in British 26 year olds: national longitudinal birth cohort. Br. Med. J. 316: 1058-1059.

Onderdonk, A., Hermos, J. and Bartlett, J. 1977. The role of the intestinal microflora in experimental colitis. Am. J. Clin. Nutr. 30: 1819.

Onderdonk, A., Hermos, J., Dzink, J. and Bartlett, J. 1978. Protective effect of metronidazole in experimental ulcerative colitis. Gastroenterology. 74: 521.

Onderdonk, A.B., and Bartlett, M.D. 1979. Bacteriological studies of experimental ulcerative colitis. Am. J. Clin. Nutr. 32: 258-265.

Onderdonk, A.B. 1983. Role of the intestinal microflora in ulcerative colitis. In: Human Intestinal Microflora in Health and Disease. D.J.Hentges, ed. Academic Press, London. p. 481-493.

Pathmakanthan, S., Thornley, J.P., and Hawkey, C.J. 1999. Mucosally associated bacterial flora of the human colon: quantitative and species specific differences between normal and inflamed colonic biopsies. Microb. Ecol. Hlth. Dis. 11: 169-174.

Pitcher, M.C.L. 1996. Sulphate-reducing bacteria, sulphur metabolism and ulcerative colitis. MD Thesis, University of Cambridge.

Pitcher, M.C.L., Beatty, E.R., and Cummings, J.H. 2000. The contribution of sulphate reducing bacteria and 5-aminosalicylic acid to faecal sulphide in patients with ulcerative colitis. Gut. 46: 64-72.

Pitcher, M.C.L., Beatty, E.R., Harris, R.M., Waring, R.H., and Cummings, J.H. 1998. Sulfur metabolism in ulcerative colitis. Investigation of detoxification enzymes in peripheral blood. Dig. Dis. Sci. 43: 2080-2085.

Poxton, I.R., Brown, R., Sawyerr, A., and Ferguson, A. 1997. Mucosa-associated bacterial flora of the human colon. J. Med. Microbiol. 46: 85-91.

Rath, H.C., Herfarth, H.H., Ikeda, J.S., Grenther, W.B., Hamm, T.E., Balish, E., Taurog, J.D., Hammer, R.E., Wilson, K.H., and Sartor, R.B. 1996. Normal luminal bacteria, especially *Bacteroides* species mediate chronic colitis, gastritis, and arthritis in HLA-B27/human $_2$ microglobulin transgenic rats. J. Clin. Invest. 98: 945-953.

Rath, H.C., Ikeda, J.S., Linde, H.J. *et al.* 1999. Varying cecal bacterial loads influences colitis and gastritis in HLA-B27 transgenic rats. Gastroenterology. 116: 310-319.

Rath, H.C., Schultz, M., Freitag, R., Dieleman, L.A., Li, F., Linde, H.-J., Schölmerich, J. and Sartor, R.B. 2001. Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice. Infect. Immun. 69: 227-2285.

Rembacken, B.J., Snelling, A.M., Hawkey, P.M., Chalmers, D.M., and Axon, A.T.R. 1999. Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: a randomised trial. Lancet 354: 636-639.

Rizzello, F., Gionchetti, P., Venturi, A., Ferretti, M., Peruzzo, S., Raspanti, X., Picard, M., Canova, N., Palazzini, E and Campieri, M. 1998. Rifaximin systemic absorption in patients with ulcerative colitis. Eur. J. Clin. Pharmacol. 54: 91-93.

Rizzello, F., Gionchetti, P., Ferrieri, A., Venturi, A., Brignola, C., Peruzzo, S., Raule, E., Descombe, J.J., Picard, M. and Campieri, M. 1997. Rifaximin, a non-absorbable antibiotic in treatment of steroid-resistant ulcerative colitis: A double-blind placebo controlled trial. Gastroenterology. 112: A1072.

Rizzello, F., Gionchetti, P., Venturi, A., Ferretti, M., Peruzzo, S., Raspanti, X., Picard, M., Canova, N., Palazzini, E. and Campieri, M. 1998. Rifaximin systemic absorption in patients with ulcerative colitis. Eur. J. Clin. Pharmacol. 54: 91-93.

Roediger, W.E.W., Duncan, A., Kapaniris, O., and Millard, S. 1993. Sulphide impairment of substrate oxidation in rat colonocytes: A biochemical basis for ulcerative colitis? Clin. Sci. 85: 1-5.

Ruseler van Embden, J.G.H., Schouten, W.R. and van Lieshout, L.M.C. 1994. Pouchitis: result of microbial imbalance? Gut. 35: 658-664.

Saitoh, S., Noda, S., Aiba, Y., Takagi, A., Sakamoto, M., Benno, Y., and Koga, Y. 2002. *Bacteroides ovatus* as the predominant commensal intestinal microbe causing a systemic antibody response in inflammatory bowel disease. Clin. Diagnost. Lab. Immunol. 9: 54-59.

Sartor, R.B., Rath, H.C. and Sellon, H.K. 1996. Microbial factors in chronic intestinal inflammation. Curr. Opin. Gastroenterol. 12: 327-333.

Satsangi, J., Landers, C.J., Welsh, K.I., Koss, K., Targan, S.R., and Jewell, D.P. 1998. The presence of anti-

neutrophil antibodies reflects clinical and genetic heterogeneity within inflammatory bowel disease. Inflamm. Bowel Dis. 4: 18-26.

Schultsz, C., Moussa, M., van Ketel, Tytgat, G.N.J., and Dankert, J. 1997. Frequency of pathogenic and enteroadherent *Escherichia coli* in patients with inflammatory bowel disease and controls. J. Clin. Pathol. 50: 573-579.

Schultsz C., van den Berg, F., Ten Kate, F.W., Tytgat, G.N.J., and Dankert, J. 1999. The intestinal mucus layer from patients with inflammatory bowel disease harbors high numbers of bacteria compared with controls. Gastroenterology. 117: 1089-1097.

Schultz, M., Veltkamp, C., and Dieleman, L.A., 2002. *Lactobacillus plantarum* 299V in the treatment and prevention of spontaneous colitis in interleukin-10-deficient mice. Inflamm. Bowel Dis. 8: 71–80.

Sellon, R.K., Tonkonogy, S., Schultz, M., Dieleman, L.A., Grenther, W., Balish, E., Rennick, D.M. and Sartor, R.B. 1998. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect. Immun. 66: 5224-5231.

Steffen, R. 2001. Rifaximin: A nonabsorbed antimicrobial as a new tool for treatment of travelers' diarrhea. J. Travel Med. 8: S34-S39.

Truelove, S.C. and Jewell, D.P. 1974. Intensive intravenous regimen for severe attacks of ulcerative colitis. Lancet. i: 1067-1070.

Turunen, U.M., Färkkila, M.A., Hakala, K., Seppälä, K., Sivonen, A., Ögren, M., Vuoristo, M., Valtonen, V.V. and Miettinen, T.A. 1998. Long-term treatment of ulcerative colitis with ciprofloxacin: a prospective, double-blind, placebo-controlled study. Gastroenterology. 115: 1072-1078.

Turunen, U.M., Saarinen, M., Farkkila, M.A., Valtonen, V.V. and Granfors, K. 1999. Antibody responses to *Escherichia coli, Proteus mirabilis* and *Klebsiella Pneumoniae* in ulcerative colitis during ciprofloxacin treatment. Gastroenterology. 116: A834.

Tvede, M., Bondesen, S., Nielsen, O.H., and Rasmussen, S.N. 1983. Serum antibodies to *Bacteroides* species in chronic inflammatory bowel disease. Scand. J. Gastroenterol. 18: 783-789.

Van der Waaij, D., Cohen, B.J. and Anver, M.R. 1974. Mitigation of experimental inflammatory bowel disease in guinea pigs by selective elimination of the aerobic gram-negative intestinal microflora. Gastroenterololgy. 67: 460-472.

Venturi, A., Gionchetti, P. Rizzello, F., Johansson, R., Zucconi, E., Brigidi, P., Matteuzzi, D., and Campieri, M. 1999. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. Aliment. Pharmacol. Ther. 13: 1103-1108.

Vermeire, S. and Rutgeerts, P. 2001. Is there a role for antibiotics in Crohn's disease? In: Challenges in Inflammatory Bowel Disease. D.P. Jewell, B.F. Warren, and N.J. Mortensen, ed. Blackwell Science, Oxford. p. 121-125.

Victor, R.J., Kirsner, J.B., and Palmer, W. 1950. Failure to induce ulcerative colitis experimentally with filtrates of feces and rectal mucosa. Gastroenterology. 14: 398.

Videla, S., Vilaseca, J., Guarner, F., Salas, A., Treserra, F., Crespo, E., Antolin, M. and Malagelada, J.-R. 1994. Role of intestinal microflora in chronic inflammation and ulceration of the rat colon. Gut. 35: 1090-1097.

Walmsey, R.S., Anthony, A., Slim, R., *et al.* 1998. Absence of *Escherichia coli*, *Listeria monocytogenes*, and *Klebsiella pneumoniae* antigens within inflammatory bowel disease tissues. J. Clin. Pathol. 51: 657-661.

Watt, J. and Marcus, R. 1973. Experimental ulcerative disease of the colon in animals. Gut. 14: 506-510.

Zinkevich, V., and Beech, I.B. 2000. Screening of sulfate-reducing bacteria in colonoscopy samples from healthy and colitic gut mucosa. FEMS Microbiol. Ecol. 34: 147-155.