

Review

Bacteria as the cause of ulcerative colitis

Ulcerative colitis (UC) is a chronic inflammatory condition of the large bowel of unknown aetiology, characterised by the presence of bloody diarrhoea and mucus associated with a negative stool culture for bacteria, ova, or parasites.

This definition finds its historical rationale in the first supposed description of the disease by Wilks and Moxon more than one century ago (1875)¹; they reported a case of bloody colitis that was apparently not caused by dysenteric pathogens. Later, Sir William Hale-White reported upon occasional patients with severe ulceration of the colon not due to tuberculosis, typhoid fever, or malignant disease. The origin remained obscure, however, and he felt this condition should not be confused with bacillary dysentery.²

Since these first descriptions, are there now data supporting a non-bacterial origin of the disease as suggested, or have we found evidence to support a bacterial role in the onset of symptoms?

In the last decade, the dogma that no bacteria could grow in the acid milieu of the stomach has been systematically destroyed by the evidence that an infective agent, *Helicobacter pylori*, is responsible for gastric/duodenal disease.³ If only a few thousand bacteria can cause gastritis, can we be so sure that among the billions of bacteria living within the colon some strains are not responsible for the onset of intestinal inflammation or for its perpetuation?

During the period 1938-1954, the only drug available for treatment of UC was sulphasalazine (SASP). Nanna Svartz used SASP, which is composed of a sulphonamide-sulphapyridine and a salicylate-5-aminosalicylic acid (5-ASA). Because of its anti-bacterial activity, it was postulated that the onset of UC might have some linkage with bacteria.⁴ Though we know today that 5-ASA is the active part of SASP,⁵ a recent meta-analysis showed a trend towards a superior efficacy of the parent compound over 5-ASA derivatives in the prevention of UC relapses.⁶

Truelove and Jewell, treating severe attacks of colitis, suggested the crucial role of the "five days intensive intravenous treatment" based on the administration of high doses of corticosteroids and antibiotics.⁷ Were these latter used just to protect the patients from possible bacterial opportunistic infections secondary to corticosteroids, or to minimise a potential pathogenic role of bacteria?

Starting from the above mentioned considerations, let's now try to answer the following questions systematically:

- is there a specific bacterial agent responsible for UC onset or relapses?
- are bacterial agents able to reproduce colitis in animal models?
- have antibiotics been useful in the management of UC?
- can UC be considered as the result of the breakdown of tolerance towards the normal colonic flora?
- could we expect some benefit by treating patients with probiotics?

Role of bacteria in ulcerative colitis

For many years, researchers have been addressing the question as to whether a specific pathogen could cause inflammatory bowel disease (IBD). For instance, much attention has been paid to the role of *Mycobacteria* in the onset of Crohn's disease (CD)⁸ and more recently it has been suggested that a particular subtype of *Escherichia coli*

could play a pathogenic role in CD.⁹ The presence of *Shigella* or *Shigella*-like toxin, *Salmonella* and *Yersinia* has been investigated as a possible cause of UC, whereas *Clostridium difficile* toxin has been associated with disease exacerbation¹⁰; a similar role has been suggested for *Salmonella* infection, perhaps associated with a diminished protective activity of the mucus.¹¹ More recently, high serum antibody titres to the outer membrane protein of *Bacteroides vulgatus* have been found in patients with UC,¹² but all these results have been rather inconclusive. As *E coli* is the predominant aerobic Gram negative species of the normal intestinal flora, much more attention has been paid to a possible role of its subtypes. Besides commensal strains, certain clones possess virulent properties and cause disease in humans; the diarrhoeagenic subtypes of *E coli* belong to this latter group, showing properties such as adherence to the gut mucosa, production of enterotoxins and cytotoxins, and tissue invasion. Six major categories of diarrhoeagenic *E coli* are distinguished: enterotoxigenic *E coli* (ETEC), enteropathogenic *E coli* (EPEC), enterohemorrhagic *E coli* (EHEC), enteroaggregative adherent *E coli* (EAaggEC), diffuse adherent *E coli* (DAEC) and enteroinvasive *E coli* (EIEC).¹³

The presence of *E coli* in patients with UC has been investigated, and it has been reported that *E coli* could be detected only in a small proportion of tissue samples.^{14 15} Studies on mucosal adhesion of pathogenic bacteria in UC are controversial. A significantly enhanced adhesion of isolates of *E coli* from stool specimens and rectal biopsies from UC patients to buccal epithelial cells was found in comparison with patients with infectious diarrhoea or normal controls. The adhesive properties were similar to those of pathogenic intestinal *E coli*, suggesting that virulent *E coli* strains might participate in the pathogenesis of UC.^{16 17} Another study reported adherence of only the DAEC and EAaggEC *E coli* subtypes to rectal mucosa, however, no differences in adhesion could be found between UC patients and controls.¹⁸ Adherence of a different strain (EHEC) has also been described.¹⁹ Using a hybridisation in situ technique, a significantly higher number of bacteria was found within the mucus layer and not adherent to the surface of the epithelium in UC patients compared with controls, independently from the degree of inflammation. It is most likely that the bacteria belong to a variety of species, when considering the broad specificity of the probe used in this study.²⁰

To summarise, there is incomplete information and continuing controversy about the role, adherent properties, and subtypes of *E coli* which might be important in the pathogenesis of UC.

Another possibility is that functionally abnormal bacteria can cause inflammation through the impairment of epithelial cell metabolism. We know that colonic anaerobic bacteria are able to break down ingested carbohydrates and proteins, through the process of fermentation, into short chain fatty acids (SCFA), which are the main source of energy for colonocytes.²¹ It has been postulated that a deficiency of this energy might lead to the onset of colitis.²² In

Abbreviations used in this paper: UC, ulcerative colitis; SASP, sulphasalazine; IBD, inflammatory bowel disease.

patients with active UC there is an overproduction of hydrogen sulphide, toxic for the intestinal mucosa by competing with SCFA, which seems to be related to an excess of sulphate reducing bacteria (*Desulfovibrio desulfuricans*) in faecal samples.^{23, 24} This theory is supported by the evidence that administration of sulphated polysaccharides (carrageenan) in guinea pigs determine a chronic colitis similar to UC²⁵ and that treatment with 5-ASA is able to reduce faecal concentration of sulphide.²⁶

At present, we can only emphasise that some bacteria do localise in mucus and might possibly act by degrading its protective structure, leading to mucosal invasion. Therefore, the unresolved question is whether chronic, recurring inflammation is the result of a persistent infection with a specific pathogen, an exaggerated exposure to resident normal luminal bacteria products because of increased intestinal permeability or alteration of mucus composition, or an abnormally aggressive immune response to luminal components.

Animal models

Until now, the role of bacteria in the pathogenesis has been shown most convincingly in animal models. A causative role for *Bacteroides species* in experimental UC has been suggested. In a carrageenan guinea pig model of experimental colitis, germ free animals did not develop colitis until after monoassociation with *Bacteroides vulgatus*.²⁷ Subsequently, it was suggested that different strains of *B. vulgatus* determined considerable differences in the inflammatory response without correlation between the sources of strains and the severity of carrageenan induced lesions. In this model, pretreatment with metronidazole prevented colitis, while administration of Gram positive organisms or coliforms were not effective. These data suggest the need for an interaction between bacteria sensitive to metronidazole and dietary sulphate. More recently, the degree of caecal inflammation in HLA-B27 transgenic rats was shown to be correlated with levels of isolates on *Bacteroides* selective medium and increased anaerobic/aerobic and *Bacteroides*/aerobic ratios.²⁸

Indirect evidence for the interaction between luminal flora and the immune system exists from studies using animal models with disruptions in immunoregulatory molecules. It has been reported that spontaneous colitis which consistently develops in knockout and transgenic murine models, does not occur when these mice are maintained in germ free conditions.^{29, 30}

Role of antibiotics in the treatment of UC

Only a few trials of antibacterial agents have been carried out in UC and results are controversial. The rationale for their use is based on the possible pathogenetic role of bacteria, supported by clinical and experimental evidence.

Vancomycin, a non-absorbable antibiotic agent against Gram positive bacteria was administered orally in patients with idiopathic colitis. No overall difference in terms of efficacy was found between the two groups after seven days, but in UC patients there was a trend towards a reduction in the need for surgery.³¹

Metronidazole, an agent effective against anaerobic bacteria, was given intravenously in severe UC as an adjunct to the intensive intravenous regimen. No benefit was observed in the group receiving metronidazole; this drug was also ineffective when given orally.³²

Tobramycin, a non-absorbable antibiotic drug directed against Gram negative bacteria, was compared with placebo in a double blind study. Eighty-four patients with an acute relapse of UC were randomised to receive oral tobramycin or placebo for one week as an adjunct to ster-

oid therapy. At the endpoint, 74% in the tobramycin group and only 43% in the placebo group obtained a clinical remission. No difference was found in long term activity.³³

Further, a combination of tobramycin and metronidazole administered intravenously together with conventional steroid treatment in acute, severe UC did not provide beneficial outcome.³⁴

In a small double blind, placebo controlled trial rifaximin (a non-absorbable wide spectrum antibiotic) given orally in severe attacks refractory to standard treatment, showed a significant improvement in nine of 14 patients in comparison with five of 14 treated with placebo by reducing stool frequency, rectal bleeding, and sigmoidoscopic response.³⁵ Recently, the role of ciprofloxacin, an antibacterial agent active against a broad spectrum of Gram positive and Gram negative microbes, has also been explored. A short course of ciprofloxacin did not increase the proportion of patients with active UC achieving remission.³⁶ In contrast, in a double blind placebo controlled trial evaluating its efficacy in the induction and maintenance of remission in patients with UC, who responded poorly to conventional therapy with steroids and mesalazine, ciprofloxacin (500–750 mg twice a day) was significantly superior to placebo (failure rate of 21% in the ciprofloxacin group and 44% in the placebo group) when given for six months.³⁷ However, the trial design and methods adopted make it somewhat difficult to accept the favourable results of this study unequivocally.³⁸

Breakdown of tolerance towards colonic flora

Mucosal tolerance is an active process by which an injurious immune response is prevented, suppressed or shifted to a non-injurious class of immune reaction. The intestine is in permanent contact with billions of bacteria (10^{10} – 10^{12} CFU/g) belonging to the normal intestinal flora, food protein, and potentially pathogenic bacteria, and has to discriminate and define selective action towards non-pathogenic and pathogenic components. The commensal bacterial flora plays an important role in nutrition and immune functions and has metabolic activity such as detoxification.³⁹ However, in immunological terms, the intestinal microflora is not part of the host. Mucosal tolerance exists in order to prevent an immune response against the body's "own" bacteria that would otherwise give rise to chronic intestinal inflammation.^{40, 41}

The mechanisms of tolerance induction to antigens from the normal intestinal flora might be mediated by T cell anergy/deletion or induction of Th2/Th3 regulatory cells.⁴² Regulatory T cells generate cytokines such as IL-4, IL-10 and TGF- β , some of which can serve as growth and differentiation factors for Th3 cells⁴³ and as a switch factor for IgA.⁴⁴

Results from mice studies support both the role of bacteria and the importance of cellular and humoral responses in the maintenance of mucosal tolerance. It has been shown that mice with targeted deletion of IL-2 or IL-10 develop colitis when reconstituted with bacteria.^{29, 30}

In a TNBS colitis model, co-addition of IL-10 or anti-IL-12 resulted in the re-establishment of tolerance to the microflora, while proliferation against foreign intestinal flora was not downregulated.⁴⁵ In addition, studies with T cell mutant mice showed that T cells play a pivotal role in mucosal tolerance, albeit that appropriate control of B cells is also required.⁴⁶

The leading hypothesis for the development of chronic intestinal inflammation is that an abnormal immune response to normal flora might be crucial. This loss of tolerance might be due to a lack of regulatory mediators or cells, or a breakdown in barrier function which allows the access of inflammatory bacterial products to the local

immune system, thereby overwhelming normal regulation. These possibilities were supported by data obtained from several studies in IBD patients, reporting an important role for T cells in the proliferative response to intestinal flora,⁴⁷ T cell mediated immune responses to different autologous and heterologous species of bacteria from intestinal flora regulated by a complex network of T cell specificity,⁴⁸ and enhanced IgG levels against cytoplasmic proteins from commensal bacteria in active IBD.⁴⁹ Abnormalities in the mucosal permeability, resulting from either environmental triggers or an intrinsic defect in barrier function, might give rise to chronic intestinal inflammation induced by normal bacterial components in a genetically susceptible host.^{50 51}

Therapeutic role of probiotics

Probiotics are living organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition.⁵²

Most of probiotics belong to a large group of bacteria, empirically designated as lactic acid bacteria (lactobacilli and bifidobacteria), that are important components of the human gastrointestinal microflora where they exist as harmless commensals. Probiotic strains must be of human origin, because some health promoting effects may be species specific. Other required properties include acid and bile resistance, ability to survive, and being metabolically active within the intestinal lumen, where they should not persist long term. Probiotic strains must also be antagonistic against pathogenic bacteria by producing antimicrobial substances, by competitive exclusion or promoting a reduction of luminal colonic pH. Obviously, they must be safe and tested for human use.⁵³ Different strains of probiotic bacteria have very different and specialised functions. Most of the data we have about probiotics come from experimental conditions and there is a lot of scepticism among researchers, mainly because the mechanisms by which probiotic bacterial strains antagonise pathogenic gastro-intestinal micro-organisms or exert other beneficial effects in the host *in vivo*, have not yet been fully defined.⁵⁴

Very few data are available on the role of probiotics in experimental and human colitis. Two studies have shown a significant decrease in lactobacilli concentration in colonic biopsies from patients with active UC.^{55 56} Oral administration of *Lactobacillus GG* has resulted in an increase in the intestinal IgA immune response in CD patients.⁵⁷ Exogenous administration of *Lactobacillus reuteri*, either as pure bacterial suspension or as fermented oatmeal soup, was shown to prevent the development of acetic acid induced colitis⁵⁸ or methotrexate induced colitis in rats.⁵⁹ This latter could be even more effectively attenuated by *Lactobacillus plantarum*. More recently, treatment with *Lactobacillus species* was able to prevent the development of spontaneous colitis in IL-10 deficient mice,⁶⁰ and *Lactobacillus plantarum* was able to attenuate an established colitis in the same knockout model.⁶¹

In two recent controlled studies, one carried out for three months⁶² and the other for one year,⁶³ patients with ulcerative colitis were given oral mesalazine or capsules containing a non-pathogenic strain of *E coli* (Nissle 1917) as maintenance treatment. No significant difference in relapse rate was observed between the two treatments. This non-pathogenic strain of *E coli* was isolated by Alfred Nissle in 1917 from the faeces of a pioneer officer who, in contrast with his companions, was not affected during an epidemic dysentery infection.⁶⁴ The mechanisms of action for the non-pathogenic *E coli* strain hypothesised in this study were blocking receptors to preventing adhesive bacteria to be established, antagonistic activity against pathogenic and non-pathogenic enterobacteria probably through

the production of antimicrobial substances and changes in pH or chemical composition of the colonic lumen.

We have recently explored another strategy, using a probiotic preparation (VSL#3) characterised by a high bacterial concentration (300 billion/g of live microorganisms) and the presence of a mixture of different bacterial species. The rationale for this approach was to try to manipulate the intestinal microflora by influencing its microbial composition through both the high number of bacteria and the possible synergistic action of the different strains. Moreover, all strains were highly resistant to bile and acid and did not degrade the mucus *in vitro*.⁶⁵ In two studies, using this probiotic preparation, in patients with UC and pouchitis in remission, a significant increase in ingested probiotic strains was found in stool of these patients together with a significant decrease of stool pH.^{66 67} In the pouchitis study, patients treated with probiotics had a much better outcome than those who received placebo.

This positive effect of VSL#3 was recently confirmed in the prevention of pouchitis onset in patients operated of ileal-pouch anal anastomosis for UC. Patients treated with VSL#3 had a significantly lower incidence of pouchitis compared with those treated with placebo during the first year after ileostomy closure.⁶⁸

With regard to the mechanism of action of VSL#3, we have also found a significant increase in tissue levels of IL-10 during administration.⁶⁹

In a recent paper, it has been proposed that probiotic agents may prevent adherence of potential pathogenic *E coli* through an enhanced expression of intestinal mucins.⁷⁰

Conclusions

Unfortunately, attempts made so far to find a causative bacteria agent for IBD, and particularly for UC, have been unsuccessful. We can only say that a specific pathogen has not been detected yet, but we cannot exclude that one or more agents is/are responsible. The reasons for such a statement could be either the inadequacy of the methods or the complexity of the colonic ecosystem. For instance, we should not forget that some commensal bacteria might become pathogenic under certain circumstances and we have also to consider that most of the bacteria live within the lumen and not necessarily enter the mucosa.

We have learned a lot from animal models and can now say confidently that if there are no bacteria, together with a genetic predisposition, colitis will not develop. Needless to say that bacteria according to ingested nutrients might produce substances able to protect the mucosa or to favour an aggressive activity. At present, the only possibility to hand is to try to manipulate the intestinal microflora by adding potential protective bacteria such as probiotics. Preliminary clinical studies have confirmed that this approach might be extremely useful. However, much more work is necessary to understand why probiotics are able to compete with aggressive bacteria and how the communication between microflora and the immune system in healthy and UC patients works.

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