Review

Current roles of specific bacteria in the pathogenesis of inflammatory bowel disease

Lucy McMullen 1, Steven T Leach 1, Daniel A Lemberg 2, and Andrew S Day 3,*

1 School of Women’s and Children’s Health, University of New South Wales, Sydney, Australia
2 Department of Gastroenterology, Sydney Children’s Hospital, Randwick, Sydney, Australia
3 Department of Paediatrics, Otago University, Christchurch, New Zealand

* Correspondence: E-mail: andrew.day@otago.ac.nz; Tel: +64-3-3640747; Fax: +64-3-3640919.

Abstract: The relevance of alterations in gut microbiota in the pathogenesis of inflammatory bowel disease (IBD) remains unclear. Currently there is conflicting evidence with regards to the roles of specific bacterial species. Escherichia coli (particularly the adherent invasive strain) are more prevalent in those with IBD and are associated with higher risk of IBD. However, the organisms are also present in healthy individuals and colonisation does not correlate with the degree of inflammation in IBD. Campylobacter concisus is more prevalent in those with IBD and higher levels of C. concisus specific IgG antibodies are found in the serum of those with IBD compared to healthy controls. Further, C. concisus has immunogenic properties that stimulate an antibody response suggesting the bacteria might trigger or exacerbate disease. Conversely most mycobacteria are unlikely to be causative as they are not present in microbial stool cultures early in disease. In various studies, Mycobacterium avium paratuberculosis has been detected both more frequently and not at all in individuals with Crohn’s disease. Similar conflict exists with respect to Yersinia enterocolitica, Bacteroides vulgatus and Helicobacter hepaticus, which are also more prevalent in IBD. However, these organisms appear more likely to contribute to disease persistence than initial disease development. This review aims to summarise the current understanding of key bacterial species implicated in the pathogenesis of IBD.

Keywords: inflammatory bowel disease; pathogenesis; Escherichia coli; Campylobacter; Mycobacterium
1. Introduction

One hundred trillion bacterial cells reside in the human gut, mainly in the colon. More than 90% of these bacteria belong to the phyla Firmicutes, Bacteroidetes, and Actinobacteria [1]. The intestinal flora play essential roles in gut function, as they contribute to host digestion and aid in many fundamental tasks such as breakdown of carbohydrates containing dietary fibres, production of short chain fatty acids and synthesis of vitamins. Changes in the microbiota can therefore influence metabolic functions and influence host wellbeing, such as susceptibility to infections [2].

Disruptions in the gut microbiota are associated with various disease states. At present, however it is unclear whether alterations in the gut microbiota are the cause or consequence of the conditions known as the inflammatory bowel diseases (IBD) [3]. These diseases are a group of chronic remitting-relapsing conditions resulting from inappropriate immune activation arising from host responses to luminal contents (such as bacteria or bacterial components) in individuals possessing particular genetic risk factors [4]. IBD encompasses two major entities: Crohn disease (CD), which involves patchy transmural inflammation in any area of the gastrointestinal tract; and ulcerative colitis (UC), which involves continuous superficial inflammation restricted to the colon [5].

Since 1913, when histologic similarities between granulomatous enteritis in humans and Johne’s disease, a chronic enteritis in ruminants caused by mycobacterium, were first reported, the search has been underway for an individual causative organism for IBD [6]. Strong clinical evidence implicating bacteria in the pathogenesis of IBD has come to light in recent years. This includes the therapeutic efficacy of antibiotics, especially in CD [7,8]; the localization of CD to intestinal segments with the highest bacterial concentrations [9]; abnormal microbial composition in CD and UC [10]; enhanced E. coli virulence in CD [11] and increased general mucosal associated bacteria in patients with IBD [12]. Considering this evidence, this focused review aims to summarise the current understanding of several specific individual organisms that have been identified as possible contributors to the pathogenesis of IBD.

2. Escherichia coli and Adherent Invasive Escherichia coli (AIEC)

As commensal organisms, E. coli play important roles in maintaining homeostasis of the gut by acting as successful competitors against anaerobes [13]. While many studies have shown increased numbers of these aero-anaerobic gram negative rods in those with IBD compared to healthy controls, E. coli is not implicated in disease unless there is a breach in the intestinal mucosal barrier or in immunocompromised hosts [7].

It has been suggested that the virulence factors of the AIEC (including type 1 pili, flagella, and outer membrane porin C) contribute to ileal lesions in CD. Adhesion by AIEC to epithelial cells occurs by attachment of type 1 pili to the CEACAM6 receptor of epithelial cells, especially in the ileum [14]. CEACAM6 receptors become overexpressed in response to Tumour Necrosis Factor (TNF) α released by macrophages that have engulfed the AIEC, thus creating a cycle of colonization and inflammation [15].

AIEC is detected more often in those with IBD than in healthy control subjects, having been isolated in 62% of patients with CD and 68% of patients with UC, compared to 6% of healthy controls (p ≤ 0.0002) [16]. Giaffer et al [16] found no difference in the adhesion index of E. coli in UC and CD, whereas Fava et al. [17] found E. coli isolated from patients with UC were less invasive
than those from patients with ileal CD. Despite differing findings regarding the adhesion factor of *E. coli* between CD and UC, both reports indicate that AIEC is more prevalent in those with IBD than in subjects without IBD. Occurrence of AIEC in IBD has been shown to be independent of disease activity, disease site, previous surgery and sulfasalazine therapy [16].

The location of *E. coli* in the gut has been a further point of research. *E. coli* has been detected within the mucous layer, underneath erosions, and in abscesses; as well as within submucosal and muscle layers in patients with CD [6]. *E. coli* has also been isolated in germinal centres of lymph follicles [6], a portal of entry for a variety of microbial agents [17]. The occasional presence of *E. coli* in the perivascular areas of the submucosal and muscle layers in patients with CD supports the interpretation that IBD is mediated by multifocal gastrointestinal infarction, after tissue damage and inflammation by AIEC or some other pathogen. Of chronically inflamed ileal resections in CD patients, 65% contained AIEC, though 22% of CD specimens that appeared endoscopically normal still contained AIEC [18,19]. Only 3.7% of colonic biopsy specimens in the same patients contained the bacteria, suggesting AIEC is more frequently detected in the ileum than in the colon.

Aside from AIEC, Giaffer et al. [16] also investigated the impact of both verotoxin releasing and haemolytic strains of *E. coli* and established that these strains had no relationship with IBD. Combining this evidence, it is widely accepted that AIEC is more prevalent in those with IBD [6,16,17]. The presence of AIEC is associated with a higher risk of IBD [7]. Nevertheless, there is no direct evidence to show a causal relationship between the number of bacteria present and inflammation. Further, the bacteria can also be identified in healthy individuals. Therefore further evidence is required to definitively establish the involvement of *E. coli* in the pathogenesis of IBD.

### 3. *Mycobacterium*

While in the past mycobacteria have been implicated as putative organisms in IBD, it is now generally accepted that this is unlikely. Early conventional stool cultures failed to detect pathogenic bacteria [20]. Fujita et al. [6] were unable to detect common pathogens such as *M. tuberculosis*, *M. avium* and *M. paratuberculosis* in tissue samples from patients with IBD. *Mycobacterium avium paratuberculosis* (MAP), however, has been the focus of much interest.

MAP is an obligate intracellular pathogen that causes spontaneous granulomatous enterocolitis in cattle and other ruminants by evading phagocytes (a condition known as Johne’s Disease), explaining the prevalence of the organism in the immunocompromised [21]. Proposed mechanisms of transmission of MAP to humans include infected milk, meat, or water [8].

In a recent meta-analysis of 47 studies, MAP was overall detected more frequently among patients with CD compared to controls [22]. However the reported detection rates ranged from 0% to 100% in patients with CD [23–25]. Notably, this meta-analysis excluded 13 studies in which MAP was not detected in any patients with CD [26]. Nevertheless, this meta-analysis provided a pooled estimate of absolute risk of 0.23 (95% CI, 0.14–0.32) for developing disease in the presence of MAP [22]. PCR assay-based studies have shown increased detection in patients with CD than in controls on many occasions [25,27,28]. Naser et al. [29] detected MAP DNA in the blood of patients with CD, though did not sample intestinal tissue for the organism, nor explored the possibility that intestinal colonisation might be secondary due to increased gut permeability or the inability of macrophages to kill organisms in CD [22].

Despite the reported detection rate being as low as 0% in CD patients, it is clear that detection
of MAP correlates with an increased risk of having IBD, suggesting MAP could be an inflammatory stimulus in at least a subset of patients [22]. However, a well-designed, 2 year prospective trial of clarithromycin, rifabutin and ethambutol (drugs known to be effective in treating MAP) did not show a sustained response in adult patients with CD [30].

The association between MAP and the pathogenesis of IBD therefore remains inconclusive. For MAP to be considered a causative organism for IBD, evidence that infection precedes the pathology is paramount. Detection of MAP in intestinal mucosal biopsies should also be extended, rather than relying only on detection of MAP DNA in the blood.

4. **Candidate Division TM7 (**Candidatus** Saccharibacteria)**

Candidate division TM7 (TM7), a phylum of gram negative bacteria, have been shown to be a cause of inflammatory mucosal diseases, particularly periodontitis [31–34]. In a 2008 study, Kuehbacher et al. [31] suggested that genetically determined antibiotic resistance of TM7 contributes to early stage IBD progression. By modifying growth conditions for competing bacterial populations, TM7 acts as a promoter of inflammation [31]. rDNA from TM7 bacteria was amplified from mucosal samples of both healthy controls and patients with active IBD with no significant difference in detection rates between the two groups. However, a significant difference in the filamentous morphotype of the bacteria was found; with the highest diversity of TM7 identified in CD (23 different operational taxonomic units), compared to 10 in UC and 12 in controls [31].

Further studies relating cause and effect must be undertaken for this subgroup of bacteria. Considering the statistically significant difference in TM7 filamentous morphotypes, identification and study of the morphotypes prominent in CD and UC should be compared directly to those in healthy individuals to make steps towards identifying how TM7 contributes to IBD pathogenesis.

5. **Campylobacter**

*Campylobacter* species have been studied in relation to IBD since 1980, when *C. jejuni* was linked to flares of disease [35]. However, more recent research has focused on the relevance of *C. concisus* to IBD.

In 2009, a significantly higher presence of *C. concisus* DNA in intestinal biopsy samples and higher levels of *C. concisus* specific IgG antibodies in the serum were detected in children with newly diagnosed CD than in controls [36]. This was supported by subsequent analysis of faecal samples using a PCR assay targeting the 16s rRNA gene of *C. concisus*, where 65% of children with newly diagnosed CD were positive for *C. concisus* compared to 33% of healthy children (p = 0.008) [37]. Additional evidence to support a link between *C. concisus* and IBD was presented in 2011, when Mukhopadhya et al. [6,38] showed that DNA from *C. concisus* was detected in significantly more adults with UC than healthy controls (33.3% versus 10.8%; p = 0.0019) [38].

The full sequence of the *C. concisus* UNSWCD strain, as reported in 2011, showed that *C. concisus* UNSWCD had a higher proportion of genes coding for membrane related components and bacterial response to stimuli, and less genes to encode intracellular components, transporter molecules and electron carriers than the gastroenteritis-associated *C. concisus* strain [39]. Further, characterization of the serum response to *C. concisus* in patients who were both diagnosed with CD and were positive for *C. concisus* by PCR showed that antibodies in the sera from the patients
recognized flagellin B, ATP synthase F1 α subunit, and outer membrane protein 18 of *C. concisus* [40]. Together these findings indicate that *C. concisus* has immunogenic properties, which stimulate antibody responses in patients with CD [41]. Considering such evidence, *C. concisus* might either trigger IBD or exacerbate disease; though further investigation is required. Other campylobacters (namely *C. gracilis, C. rectus,* and *C. showae*) have been detected only in children with CD, though the clinical relevance of these bacteria remains unknown [37].

6. **Bacteroides vulgatus**

*B. vulgatus* has been observed to cause colitis in animal models [42–44]. Subsequently, its potential role as a causative agent for IBD has been investigated.

In sampling of surgically resected human tissue, *B. vulgatus* was detected in all of a group of 27 patients with IBD (both UC and CD), regardless of the severity of inflammation present [6]. *B. vulgatus* was found in the mucous layer, beneath erosions, in necrotic ulcer bed tissues and abscesses. In patients with CD, *B. vulgatus* were found in the subserosal layer, in accordance with the transmural inflammation occurring in CD. Histological findings also showed that the colonic mucosal barrier was depleted due to ulceration and inflammation, and the authors suggest that this bacterium is involved in the persistence of disease by preventing or delaying remission [6]. However, the relationship between number of bacteria present and degree of inflammation was inconsistent [6]. Further research, including evidence of the absence of *B. vulgatus* before clinical onset of IBD is necessary to attribute a direct role for this bacterium in the pathogenesis of IBD.

7. **Yersinia**

*Yersinia* are fastidious, Gram-negative coccobacilli found in meat, dairy products, vegetables, and water, and have previously been implicated in appendicitis, ileitis and colitis [45].

Kallinowski et al. [20] reported presence of *Yersinia* in 63% of 21 patients with CD, 48% of 14 with UC and 36% of 24 healthy, disease free controls. These authors concluded that *Yersinia* species appeared to persist in the intestinal tissue of patients with IBD without an adequate immune response, thereby contributing to tissue destruction. Following this, a total of 54 intestinal resection specimens from 52 patients with confirmed CD were analysed by PCR for *Yersinia* DNA and compared to disease controls [45]. Seventeen of 54 (31%) samples contained *Yersinia* (9 were positive for *Yersinia enterocolitica*, 6 were positive for *Yersinia pseudotuberculosis*, and 2 were positive for both), while all control specimens were negative. Several other authors have also demonstrated a relationship between *Yersinia enterocolitica* and IBD, and have suggested it as a possible cause for the disease [45,46]. However, a further report showed no evidence of *Yersinia enterocolitica* in biopsies from 56 patients with CD [47].

At present, therefore, no conclusions can be made as to whether *Yersinia* is a causative organism of IBD. The possibility remains that *Yersinia* is a causative agent in at least a subset of IBD patients. One difficulty, however, is the pathological and clinical overlap between CD and the inflammatory state of yersiniosis itself.
8. **Listeria monocytogenes**

*Listeria monocytogenes*, a human pathogen found in foods, preferentially invades M cells overlying lymphoid aggregates in the intestine, thus producing ileocolitis [48]. In 1995, for the first time, *L. monocytogenes* was identified in macrophages and giant cells in the involved mucosa of 75% of a group of patients with CD, but just 13% in a group of patients with UC, and in none of the 10 control subjects [49].

In 2000, DNA was extracted from 274 colonic biopsies from 23 patients with CD, 28 with UC, and 36 controls [50]. PCR amplification was used to investigate the possible relationship between *L. monocytogenes* and IBD, where it was detected in 6% of patients with CD, 5.3% of patients with UC, and 17.7% of non IBD controls [50]. The detection of the bacteria in patients with and without IBD is reflective of the widespread nature of the organism in the environment, though the low yield of positive biopsies in IBD patients compared to non IBD patients does not support a causative role in the pathogenesis of IBD [50]. Combining this, and the fact that 83% of CD patients with positive results in the 1995 study [49] had coexistent *E. coli* infection (suggesting non-specific secondary invasion), it is highly unlikely that *L. monocytogenes* is a causative organism for IBD.

9. **Helicobacter hepaticus**

In a murine study, infection with *H. hepaticus* induced IBD-like lesions in severely immunocompromised mice, and initiated rapid development of colitis and large bowel carcinoma [51,52]. However, in immunocompetent mice, *H. hepaticus* failed to induce significant disease [51]. Subsequently disparate results have been reported with a positive correlation between *H. hepaticus* and disease in TCRαβ mutant mice [53], and a negative correlation in interleukin-10-deficient mice [54]. Therefore, *H. hepaticus* was identified as a possible causative pathobiont (an organism that causes disease only in the immunocompromised setting) that is able to promote colitis in mouse strains with disrupted immune function [51].

The current evidence implies that *H. hepaticus* is more likely to be involved in disease persistence than causation (as IBD induces an immunocompromised state), though further human studies are necessary.

10. **Strentrophomonas maltophilia**

In 2009, archival intestinal resection samples from 56 patients with CD were investigated for the presence of pathogenic bacteria [47]. Resections were positive for *Strentrophomonas maltophilia* (found in 17.9% of CD patients) [47], a pathogen recognized in many other inflammatory conditions such as bacteremia and urinary tract infections [55,56]. Although the authors made no conclusions regarding the role of this organism in IBD, the presence of known pathogens in disease tissue requires further investigation.

11. Conclusion

While several bacteria have been implicated as possible causes for IBD, available data has not yet identified a single, causative organism for IBD. MAP and *E. coli* are detected at particularly high
rates in patients with IBD, and may play roles in IBD pathogenesis. Data regarding the roles of other putative organisms are currently inconclusive.

Integration of information regarding possible bacterial pathogens with host genetic polymorphisms and gene expressions will increase our understanding of microbial and host interactions. As many as 25% of patients with CD have mutations in the NOD2/CARD15 gene on chromosome 16 [57]. It has been suggested that the NOD2/CARD15 product increases likelihood of CD development by altering bacterial recognition components [57]. Furthermore, using this information, prospective studies might provide insight into the level of inflammation with each new putative pathogenic bacterium.

The advent of increasingly sophisticated molecular tools should enable further advances in our understanding of the role that these, or other, microbial agents play in the development or perpetuation of IBD. In addition, studies such as the GEM project (www.gemproject.ca) focusing on first degree relatives at risk of developing IBD over time, may also lead to better understanding. Furthermore, additional endeavours are required to distinguish between the relevance of individual organisms (including those included here), the importance of alterations in the overall patterns of the intestinal microbiota (dysbiosis) and the loss of beneficial species such as \textit{Faecalibacterium prausnitzii} and how each of these microbial factors contributes to the pathogenesis of IBD.

Conflict of Interest

The authors declare no conflict of interest in this research.

References


