An overview of the bacterial contribution to Crohn disease pathogenesis

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Crohn disease (CD) is a chronic inflammatory condition primarily affecting the gastrointestinal tract and is characterized by reduced bacterial diversity. The exact cause of disease is unknown; however, evidence suggests that several components, including microbiota, may contribute to the underlying pathology and disease development. Perturbation of the host–microbe commensal relationship is considered the main driving force of tissue destruction and pathological changes seen in CD. Several putative bacterial pathogens including species from Mycobacterium, Campyllobacter and Helicobacter are postulated in the aetiology of CD. However, to date, no strong evidence supports a single bacterium contributing overall to CD pathogenesis. Alternatively, dysbiosis or bacterial imbalance is more widely accepted as a leading factor in the disrupted host–immune system cross-talk resulting in subsequent intestinal inflammation. Depletion of symbiont microbes including Firmicutes, Bifidobacterium and Clostridia, in conjunction with an increase in pathobiont microbes from Bacteroidetes and Enterobacteria, is a striking feature observed in CD. No single factor has been identified as driving this dysbiosis, although diet, antibiotic exposure and possible early life events in presence of underlying genetic susceptibility may contribute. The aim of this review is to highlight the current accumulating literature on the proposed role of bacteria in the pathogenesis of CD.

Introduction

Crohn disease (CD) is one entity of the group of conditions termed the inflammatory bowel diseases (IBD) (Leach & Day, 2006). CD is a complex heterogeneous disorder with the underlying aetiopathogenesis being multifactorial with genetic, immunologic, environmental and microbial factors contributing to the disease (Alhagamhmad et al., 2012). Evidence of the role of bacteria in CD pathogenesis was historically provided by the successful role of antibiotics in CD treatment (Greenbloom et al., 1997), with additional evidence provided by the success of faecal diversion in preventing disease relapse (Rutgeerts et al., 1991). However, precisely how bacteria contribute to pathogenesis remains incompletely understood. Nevertheless, there is a consensus that the intestinal microbiome is a key factor in the development and maintenance of mucosal homeostasis, and loss of that function contributes to intestinal inflammation (Buttó et al., 2015).

The intestinal microbiota

Microbiota concepts

A large number of micro-organisms reside in niches adjacent to epithelial surfaces of the human body with the majority of these organisms colonizing the intestine, and collectively, these organisms are known as the intestinal microbiota (Palmer et al., 2007; Marchesi et al., 2016; Lozupone et al., 2012). Recent advances in bacterial detection methods, most notably culture-independent molecular-based technologies, have resulted in a deeper understanding of the structure and function of the human intestinal microbiota (Cocolin et al., 2013; Hiergeist et al., 2015). Historically, culture-based plating techniques were the primary method of examining the intestinal microbiota. These techniques were subsequently superseded by conventional DNA-based techniques using sequencing or
fluorescent detection; however, only a limited proportion of microbiota was successfully detected resulting in an incomplete picture of gut–microbial interactions (Hiergeist et al., 2015). Culture-independent detection methods have now progressed to RNA-based profiling (16S rRNA gene sequencing) (Olsen et al., 1986) and, more recently, high-throughput DNA sequencing (Metzker, 2005) and third-generation sequencing analysis (Weinstock, 2012). Utilizing these recent advents, the whole human microbiome can now be rapidly sequenced, resulting in highly detailed classifications as well as the compositions of functional genes present in the microbiome (Lozupone et al., 2012; Turnbaugh et al., 2007). These techniques allow for characterizing disease-associated microbiota changes, together with precisely specifying altered individual microbial species (Srinivas et al., 2013; Ley et al., 2008; Sekirov et al., 2010).

Bacteria account for largest contribution to the intestinal microbiome, although Archaea, viruses, fungi and protozoa are also present (Tlaskalová-Hogenová et al., 2011; Hoffmann et al., 2013). The human gut-associated bacteria are predominantly derived from four predominant phyla: Firmicutes, Bacteroidetes, Actinobacter and Proteobacteria (Tlaskalová-Hogenová et al., 2011; Clemente et al., 2012; Turnbaugh et al., 2009). However, between individuals, there is a significant diversity in composition within these phyla, with genetics, diet, the environment and host gender implicated as putative factors contributing to the diversity (Koboziev et al., 2014). Despite the diversity, in health, the commensal bacteria contribute vital functions including fundamental roles in nutrition, metabolism, cancer prevention and resilience to pathogens (Flint et al., 2012). In addition, the microbiota are key to the development and maintenance of mucosal and systemic homeostasis (Koboziev et al., 2014). Animal studies have shown that mice raised in germ-free conditions can be affected by improper maturation of gut immunity characterized by abnormal T- and B-cell functions, rudimentary lymphoid tissues, lower levels of circulating CD4+ T cells and impaired antibody production (Chung et al., 2012). Interestingly, these changes can be reversed upon restoration of commensal bacteria (Chung et al., 2012).

**Microbiota–immune system cross-talk**

The interaction of commensal bacteria with the immune system continues to be an area of interest. Various mechanisms have been described through which the intestinal microbiota may interact with mucosal immunity (Hooper et al., 2012; El Aidy et al., 2015). One key pathway is toll-like receptor (TLR) signalling, which can influence regulatory T-cell populations, the autophagy pathway (Sun et al., 2011; Frosali et al., 2015), cellular epithelial proliferation, cytotoxic effects, immune cell recruitment, IgA production and secretion of antimicrobial peptides (Rakoff-Nahoum et al., 2004; Shang et al., 2008; El Aidy et al., 2015). It is also increasingly evident that enteric bacteria influence production of effector CD4+ T helper (Th) cells, which are imperative in protecting the gut from developing colitis (Kullberg et al., 2002; Kamada & Núñez, 2014). Certain species of segmented filamentous bacteria induced production of Th17 cells, which protects the host from bacterial infections via the secretion of IL-17, IL-21 and IL-22 (Ivanov et al., 2009). The Th17 cells also contribute to intestinal homeostasis by regulating intestinal polymeric Ig receptor expression and IgA secretion (Cao et al., 2012). Clostridia strains including clusters IV, XIVa and XVIII have also been shown to enhance the abundance of intestinal CD4+Foxp3+ regulatory T cells (Atarashi et al., 2013), which perform several functions including suppressing exaggerated immune responses to bacterial antigens, enhancing epithelial barrier integrity and increasing production of the anti-inflammatory cytokine IL-10 (Sun et al., 2011; Koboziev et al., 2014).

The intestinal mucosa can also influence the microbiota through detecting and destroying translocated microbes (Hooper et al., 2012) and via production of specific antimicrobial peptides including defensins, cathelicidins, lipocalin-2 and cathepsin K (Koboziev et al., 2014). In health, these interactions develop into a homeostatic relationship between the microbiota and the mucosa (Pagliari et al., 2015), and it is the disruption of this homeostasis that appears to be a key element in developing colitis (Öyri et al., 2015).

**Intestinal microbiota and dysbiosis**

**Dysbiosis concepts and IBD**

Dysbiosis is an alteration and disturbances in the normal observed diversity of gut microbiota and is associated with numerous diseases including obesity, diabetes and IBD (Casen et al., 2015). Dysbiosis is considered one of the abnormal prominent features of the bacterial changes accompanying CD (Manichanh et al., 2006; Seksk et al., 2003; Chamaillard & Radulovic, 2016) (Table 1). Indeed, several studies have documented a significant difference in the microbiota composition of healthy individuals compared to those with IBD (Marchesi et al., 2007; Zhang et al., 2007; Qin et al., 2010; Wright et al., 2015). Although there is an overlap in dysbiotic changes accompanying CD and ulcerative colitis (UC) (Frank et al., 2007), it should be noted that there is a considerable difference in the composition of mucosal and faecal microbial communities between the two disease entities (Tamboli et al., 2004; Walker et al., 2011; Sadowsinski et al., 2008). Frank et al. (2007) have reported a decrease in the abundance of 16S rRNA sequences of Firmicutes and Bacteroidetes together with an increase in Proteobacteria and Actinobacteria sequences in mucosal biopsies collected from IBD patients compared to healthy controls. However, they also reported no differences in either mucosal or faecal bacterial composition between CD and UC patients (Frank et al., 2007). In contrast, Walker et al. (2011) utilizing a full-length bacterial 16S rRNA sequencing of mucosal biopsies taken from IBD patients and healthy controls reported considerable discrepancy in
the microbiota compositions amongst the IBD disease entities. *Firmicutes* were reduced in all IBDs, together with increases in *Bacteroidetes*; however, the abundance of *Enterobacteriaceae* was elevated only in CD (Walker et al., 2011).

**Dysbiosis in CD**

It has been reported that around one-third of dominant species in the typical CD microbiome belongs to new phylogenetic groups not typically dominant in healthy individuals (Seksik et al., 2003). The most defined changes encompass depletion in *Firmicutes* in conjunction with an increase in *Proteobacteria* (*Escherichia coli* and *Bacteroides*) (Loh & Blaut, 2012) (Table 1). Amongst the *Firmicutes*, a reduction in the abundance of the commensal *Faecalibacterium prausnitzii* in CD patients has been well documented (Sokol et al., 2009; Fujimoto et al., 2013). *F. prausnitzii* inhabits the gut mucosa and mediates anti-inflammatory activities through production of a protein with immunomodulating properties (Quévrain et al., 2015). Furthermore, there is a strong association between depletion of *F. prausnitzii* and the disturbed immune tolerance to the intestinal microbiota (Sokol et al., 2008). Further reports also indicate a reduction in faecal lactobacilli and bifidobacteria in CD patients (Favier et al., 1997). In regard to specific species, *Dialister invisus* and *Clostridium* clusters were also found to be fewer in CD than in matched healthy controls (Joossens et al., 2011). *Clostridia*, particularly IV and XIV, are the main butyrate-producing bacteria in the gut (Fava & Danese, 2011). Butyrate is a major fuel source for colonic epithelial cells (Fleming & Floch, 1986) and exerts anti-inflammatory activity through suppression of NFκB signalling (Segain et al., 2000). Butyrate is also required for maintaining epithelial barrier integrity (Vanhoutvin et al., 2009); thus, loss of clostridial clusters is likely to contribute to loss of vital epithelial barrier functions (Nagalingam & Lynch, 2012).

In addition to depletion of beneficial microbiota, a relative increase in the abundance of potentially pathogenic species in CD has also been documented (Frank et al., 2011; Lupp et al., 2007) (Table 1). While the role of these species in disease pathogenesis has not been confirmed, *Bacteroidetes*, Enterobacteria, *Ruminococcus gnavus* and *Pseudomonas* have been observed more frequently in CD patients (Walker et al., 2011; Joossens et al., 2011; Seksik et al., 2003; Scaldaferrti et al., 2013). Enterococcal species, *Clostridium perfringens* and *Bacteroides fragilis*, were specifically isolated more often from the mesenteric lymph nodes of surgically treated CD patients (De Hertogh et al., 2008). In a study comparing the abundance of bacteria in mesenteric lymph nodes in CD and healthy controls, it was noted that these species were identified in approximately 40% of CD patients compared to only 10% of a healthy control group (Matricon et al., 2010). Thus, the shift from predominant 'symbiont' microbes to potentially harmful 'pathobiont' microbes may drive a breakdown of host–microbial cross-talk that likely leads to further changes in microbiota composition. Furthermore, this shift in microbiota composition is considered to stimulate high loads of bacterial antigens capable of perpetuating an inflammatory response (Abraham & Medzhitov, 2011).

It should be noted that dysbiotic signature characterizing CD can be observed in both the faecal and mucosal microbiota communities (Manichanh et al., 2006; Frank et al., 2007). In one study by Joossens et al. (2011), faecal samples were collected from CD patients and analysed using denaturing gradient gel electrophoresis. The authors found a decrease in several species including *Firmicutes* (*F. prausnitzii*), together with an increase in *R. gnavus* (Joossens et al., 2011). In a further study using 16S rRNA sequencing of mucosal biopsies from CD patients, diversity of the mucosal microbiota was reduced, and the species composition was disturbed (reduction in *Firmicutes* with concurrent increases in *Enterobacteriaceae*) (Walker et al., 2011).

**Dysbiosis and disease relapse**

An emerging topic of interest is the investigation of dysbiosis in predicting CD relapse. Microbial patterns in patients with recurrent disease have been demonstrated to have reduced abundance of *Firmicutes*, in particular, *F. prausnitzii* (Sokol et al., 2008), as well as increased adherent invasive *E. coli* (AIEC) (Lepage et al., 2009; Darfeuille-Michaud et al., 2004). In a study exploring changes in the microbiota profile of 12 patients with CD at the time of surgical intervention, biodiversity was lower at the time of surgery and

Table 1. Dysbiotic changes in the intestinal microbiome in CD compared to healthy controls

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Comment</th>
<th>References</th>
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<tr>
<td><em>Firmicutes</em> (<em>F. prausnitzii</em>)</td>
<td>Decrease</td>
<td>Fujimoto et al. (2013)</td>
</tr>
<tr>
<td><em>Bifidobacterium</em> (<em>Bifidobacterium adolescentis</em>)</td>
<td>Decrease</td>
<td>Joossens et al. (2011)</td>
</tr>
<tr>
<td><em>Clostridium</em> clusters (IV and IXV)</td>
<td>Decrease</td>
<td>Fava &amp; Danese (2011)</td>
</tr>
<tr>
<td><em>D. invisus</em></td>
<td>Decrease</td>
<td>Joossens et al. (2011)</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>Decrease</td>
<td>Favier et al. (1997)</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em> (<em>E. coli</em>)</td>
<td>Increase</td>
<td>Seksik et al. (2003)</td>
</tr>
<tr>
<td><em>R. gnavus</em></td>
<td>Increase</td>
<td>Joossens et al. (2011)</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>Increase</td>
<td>Scaldaferrti et al. (2013)</td>
</tr>
<tr>
<td><em>Bacteroidetes</em></td>
<td>Increase</td>
<td>Walker et al. (2011)</td>
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was increased 6 months after surgery (De Cruz et al., 2015). However, at 6 months following surgery, biodiversity still remained different in healthy subjects (De Cruz et al., 2015). This study also found that patients with recurrent disease had a predominance of Enterococcus, whereas those who maintained remission showed predominance of butyrate-producing Firmicutes (De Cruz et al., 2015). Similar observations were also reported by Rajca et al. (2014) in a recent cohort study that explored microbiota changes in 33 patients with CD. Low counts of Firmicutes including F. prausnitzii were correlated with higher rates of disease relapse (Rajca et al., 2014). More interestingly, the study showed that F. prausnitzii in particular predicted the occurrence of relapse independently of raised inflammatory markers. Thus, it appears that monitoring patients’ microbiota might provide a new prognostic tool for assessing risk of relapse and/or the recurrence following surgery in CD. However, further investigations are required to determine the sensitivity and specificity of such markers for disease relapse.

Dysbiosis and the dysregulated immune response

There is a consensus that interaction of several putative factors, including microbial, genetic and environment, contributes to loss of mucosal homeostasis, the subsequent provocation of the mucosal immune system and development of CD (Chu et al., 2016; Lewis et al., 2015) (Fig. 1). However, the microbial contribution appears to be the only consistent factor for all cases of CD. Bacteria can activate both the innate (macrophage, neutrophil and dendritic cells) and acquired (T and B cell) immune systems (Sartor, 2006; Hooper et al., 2012; Alexander et al., 2014; Kayama & Takeda, 2016). Bacteria can act as adjuvants to activated innate immune cells or as antigens to stimulate clonal expansion of T cells (Sartor, 2007; Frosali et al., 2015; Tanoue et al., 2016). Innate immunity recognizes microbe-associated molecular patterns by specific receptors including TLRs, C-type lectin receptors, nucleotide-binding oligomerization domain (NOD) and sense pathogen motifs (Tkalskalová-Hogenová et al., 2011; Ignacio et al., 2016; Man et al., 2016). Microbial adjuvants including LPS, peptidoglycan and flagella can bind TLRs on innate immune cells and intestinal epithelial cells and activate NFκB and the mitogen-activated protein kinases (Hruz et al., 2009; Pandey et al., 2015; Christophi et al., 2012), which are key intracellular signalling pathways governing pro-inflammatory and regulatory gene transcription. Once activated, innate immune cells stimulate the production of IL-1β, TNF, IL-6, IL-8, IL-12 and IL-23, adhesion molecules, IL-18, reactive oxygen species, nitric oxide and leukotrienes (Zareie et al., 2001; Mahida, 2000; de Zoeten & Fuss, 2013). High levels of these pro-inflammatory molecules promote trafficking and migration of the circulating immune effector cells to the intestinal mucosa (Reaves et al., 2005; Griffith et al., 2014; Peterson & Artis, 2014). Migrated monocytes and polymorphonuclear cells, rather than the local mucosal immune cells, become the main contributors in stimulating the inflammatory process seen in IBD (Sartor, 2006; Brazil et al., 2013; Fournier & Parkos, 2012). Therefore, the bacterial contribution to IBD pathogenesis can be described as a dysbiotic intestinal microbiota that is overwhelmed with immune-stimulating bacteria and depleted in immune-suppressing bacteria.

Risk factors for dysbiosis

Dietary habits

Several perinatal and postnatal factors including diet habits and early life exposure to drugs and vaccines have been claimed to be environmental factors associated with the increase in immunological diseases including CD (Alhagamhmad et al., 2015). Numerous reports demonstrate that environmental triggers, most notably diet, have profound effects on the gut microbiome and can influence microbiota composition, growth and shapes (Koropatkin et al., 2012; Carbonero et al., 2012; Sekirov et al., 2010). The influence of dietary components on the composition of the microbiota is observed during the initial colonization of newborn infants (Harmsen et al., 2000; Koenig et al., 2011). Breastfed infants have higher levels of bifidobacteria with less abundance of Bacteroides and Clostridium coccoides compared to formula-fed infants (Fallani et al., 2010). Beyond exclusive breastfeeding in the neonatal period, the gut microbiota undergoes dramatic changes until stabilizing around 3 years of age (Mackie et al., 1999). However, intestinal microbiota composition is not rigid, and the influence of diet continues throughout life (Claesson et al., 2012). Microbial alterations due to diet include a change in the diversity (Scott et al., 2013). Under normal physiological conditions in healthy individuals, pathobiont bacterial
species coexist with the commensal beneficial bacteria, albeit typically in very low abundance (Chow & Mazmanian, 2010). Certainly, Firmicutes and Bacteroidetes account for 90 % of gut bacteria (Ley et al., 2008), whereas Actinobacteria and Proteobacteria represent less than 5 % (Eckburg et al., 2005). Dietary manipulation, for example, with prebiotics (non-digestible food ingredients that promote targeted growth of beneficial micro-organisms) (Schrezenmeir & de Vrese, 2001), has been shown to modify the intestinal microbiome by promoting growth and metabolic activity of the commensal bacterial species (Macfarlane & Macfarlane, 2013). This approach is of importance in treating and/or preventing chronic inflammatory diseases including IBD (Saez-Lara et al., 2015). Prebiotics were found to alter microbial diversity, enhance mucosal barrier function via production of short-chain fatty acids and mediate direct anti-inflammatory responses (Hold et al., 2014).

In contrast, several dietary components have been found to negatively influence the composition of the microbial community and induce dysbiosis (Martins dos Santos et al., 2010; Brown et al., 2012). Both over-nutrition, seen more often in Western societies, and under-nutrition, predominant in the underdeveloped countries, are leading factors for dysbiosis (Devkota & Chang, 2013). Western diets rich in sugar and fat have been associated with overgrowth of Firmicutes, including Clostridium innocuum, Eubacterium dolichum and Catenibacterium mitsuokai (Turnbaugh et al., 2009). Refined-sugar-containing diets have also been shown to increase overgrowth of the opportunistic pathogens Clostridium difficile and C. perfringens (Brown et al., 2012), whereas diets rich in complex carbohydrates promote increased levels of beneficial bifidobacteria (Pokusaeva et al., 2011). Western-diet-induced dysbiotic changes might explain, at least in part, the association of lifestyle with increased IBD susceptibility, particularly in developed countries where there is a high prevalence of CD (Leone et al., 2013) and in areas with a recent rapid rise in CD incidence that has been attributed to westernization of dietary patterns (Ng et al., 2013).

In addition to setting of over-nutrition, under-nutrition also has profound and sustained effects on the gut microbial composition (Hashimoto et al., 2012). It has been suggested that malnutrition delays the normal assemblage of the gut microbiota in early childhood and obligates towards lack of diversity (Kane et al., 2014). However, it remains unknown whether the microbial changes accompanying under-nutrition can also lead to initiation of intestinal inflammation and subsequent IBD development. Nevertheless, preliminary observations of under-nourished mice with specific derangements in amino acid component have shown an association (Devkota & Chang, 2013). Overall, it is increasingly evident that certain dietary lifestyles and specific dysbiotic changes are emerging as likely candidates responsible for and/or that predispose individuals to IBD development.

**Antimicrobial therapy**

Antibiotic exposure has the potential to alter the gut microbiota composition (Pérez-Cobas et al., 2013). A short course of ciprofloxacin, a commonly prescribed antibiotic, induced profound and rapid changes with a loss of diversity in the intestinal microbiota composition (Dethlefsen & Relman, 2011). Furthermore, 1 week after the completion of the antibiotic course, the intestinal microbial community remained altered compared to prior to the antibiotic course (Dethlefsen & Relman, 2011). Similarly, a 10 day course of amoxicillin led to a major shift in the main components of the faecal microbiota from Bacteroides, Clostridium clusters IV and XIVa and Bifidobacterium towards an overgrowth of Enterobacteriaceae (Young & Schmidt, 2004).

Antibiotic exposure in early life predisposes to an increased risk of the development of CD (Virta et al., 2012). In a meta-analysis that included 11 observational studies, antibiotic use was associated with the potential development of CD (Ungaro et al., 2014). Case–control analysis of a population-based study involving IBD patients also reported that use of antibiotics 2–5 years prior was significantly associated with higher chance of developing CD and UC (Shaw et al., 2011). More interestingly, the study also showed a dose-dependent association between antimicrobial prescriptions and increased risk of development of IBD (Shaw et al., 2011).

However, there is debate whether dysbiosis precedes or follows IBD pathology (Buttò & Haller, 2016). With the limited available knowledge, the association of antibiotic exposure and dysbiosis, along with the increased incidence of CD, suggests that microbial dysbiosis is likely present prior to the initiation of inflammation and subsequent disease development. Thus, limiting exposure and over-prescription of antimicrobial agents, especially to those with a family history of IBD, might be of value in reducing the rising incidence of CD.

**Smoking**

Smoking, a well-known environmental risk for CD, has also been found to alter microbiota composition and induce locus mutations resulting in failure of bacterial recognition and mishandling (Sheehan et al., 2015). Interestingly, upon cessation of smoking, the microbial changes can be reversed in favour of increasing abundance of symbiont Firmicutes and a decrease in the abundance of pathobiont Bacteroidetes (Biedermann et al., 2014).

**Single causative bacteria in CD pathogenesis**

**Mycobacterial species**

An individual pathogenic organism as a single cause for IBD has also been proposed with a number of candidate bacteria identified (McMullen et al., 2015) (Table 2). Mycobacterial species including infection with Mycobacterium
avium subsp. paratuberculosis (MAP) is commonly postulated in the aetiology of CD (Greenstein, 2003; McMullen et al., 2015). In immunodeficient mice, it has been shown that MAP organisms are able to invade the gut epithelium and induce tissue damage (Golan et al., 2009). MAP has also been cultured from patients with CD, but the accuracy of such findings is still questionable (Sekskis et al., 2006). Dalton et al. (2014) investigated the associations between MAP and single nucleotide polymorphisms linked with CD in a cohort of 84 MAP-positive CD patients; however, no evidence of associations was identified. Additionally, no correlation was also found between NOD2 polymorphism and MAP serology in CD patients (Bernstein et al., 2007).

**Helicobacter species**

A role for Helicobacter species in IBD has also been proposed (Papamichael et al., 2014) (Table 2). However, findings are varied and, to date, have failed to support a definitive association in IBD (Hold et al., 2014). A recent meta-analysis involving 33 studies that included 4400 IBD patients showed a significant negative association between H. pylori and IBD (Rokkas et al., 2015). Furthermore, a recent cohort study indicated that the organism might be protective against CD development (Bartels et al., 2016).

**Proteobacteria**

E. coli and non-jejuni Campylobacter species have also been linked with IBD pathogenesis (Mukhopadhyya et al., 2012) (Table 2). AIEC has been identified in the intestinal mucosa of CD patients, specifically associated with the ileal mucosa (Darfeuille-Michaud et al., 2004). Interestingly, antibody positivity to AIEC is more prevalent in CD patients and is associated with disease severity, rapid disease progression and the increased need for surgical intervention (Hold et al., 2014). Campylobacter concisus was also isolated from intestinal biopsies and faecal samples of newly diagnosed paediatric CD patients (Man et al., 2010). A higher prevalence of other non-jejuni Campylobacter species including Campylobacter hominis, Campylobacter ureolyticus, Campylobacter showae, Campylobacter gracilis and Campylobacter rectus were also found in faecal samples of CD patients compared to matched healthy controls (Man et al., 2010). Furthermore, immune reactivity to C. concisus flagellin B, ATP synthase F1 alpha subunit and outer membrane protein 18 were recently identified in CD patients suggesting that the pathogen may be colonizing and, therefore, contributing to disease development (Kovach et al., 2011). The precise contribution of C. concisus in the development of IBD remains yet to be fully described; however, increasing intestinal permeability has been proposed as a leading factor (Zhang et al., 2014).

**Other putative bacterial pathogens**

Staphylococcaceae, Streptococcaceae, Pseudomonas maltophilia, Klebsiella and Salmonella have also been implicated in disease pathogenesis and/ or relapse (De Hertogh et al., 2008; Hold et al., 2014) (Table 2). However, these organisms are not specific to IBD and can be isolated in other conditions (Ehsani et al., 2015). Overall, several bacterial species have been isolated at higher frequency but not consistently from CD patients. However, conclusive evidence that a single bacterium contributes to disease pathogenesis, therefore, remains lacking.

**Interaction between bacteria and genetics**

**CARD15/NOD2 gene**

There is evidence of genetic contribution to CD pathogenesis where more than 160 genetic loci have been identified as risk factors for IBD (Brant, 2013), with 71 specifically associated with CD (Amre et al., 2012; Wang et al., 2013). Amongst the genetic loci implicated in CD pathogenesis, the CARD15/NOD2 gene provides the strongest contribution. NOD2 is primarily involved in bacterial clearance through binding muramyl dipeptide (MDP) to produce a number of antimicrobial peptides including defensins and mediates immune responses through activation of NfκB (Hruz et al., 2009; Dickson, 2016). Mutant CARD15 was found to be associated with defective clearance of invasive bacteria, accumulation of luminal and mucosal enteric flora and an exaggerated immune response owing to over-activation of NfκB (Sartor, 2006; Frosali et al., 2015). CARD15 mutants including Arg702Trp, Gly908Arg and Leu1007fsins C were found at higher rates in families affected with CD and are associated with a 2–4-fold relative risk increase of CD in heterozygous individuals and to 20–40-fold

**Table 2. Individual bacterial species as a single causative agent implicated in the pathogenesis of CD**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Comment</th>
<th>References</th>
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<tr>
<td>Mycobacterial species (MAP)</td>
<td>Detected more frequently in CD patients with possible inflammatory stimulus</td>
<td>McMullen et al. (2015)</td>
</tr>
<tr>
<td>Helicobacter species (H. pylori)</td>
<td>Protective role against development of CD</td>
<td>Bartels et al. (2016)</td>
</tr>
<tr>
<td>Proteobacteria (E. coli and non-jejuni Campylobacter)</td>
<td>Implicated in ileal diseases of CD by breaching intestinal barrier and increasing the permeability</td>
<td>Zhang et al. (2014) and Martin et al. (2004)</td>
</tr>
<tr>
<td>Staphylococcaceae, Streptococcaceae, P. maltophilia, Klebsiella, Salmonella</td>
<td>Causative agents have a role in the pathogenesis and relapse of CD, although they are frequently detected in other conditions</td>
<td>De Hertogh et al. (2008) and Hold et al. (2014)</td>
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relative risk increase in homozygous individuals (Li et al., 2004; Cuthbert et al., 2002).

**Atg16l1**

Additional CD susceptibility genes include ATG16L1 and immunity-related GTPase M (IRGM) genes which are involved in bacterial clearance, innate immunity and activation of adaptive immunity (Nguyen et al., 2013). ATG16L1 mutations, in association with NOD2, are associated with alterations in xenophagy activation, disrupted mucosal immune response and Paneth α-defensin exocytosis (Igor’V & Andreev, 2014). The mutation in the ATG16L1 gene that causes the amino acid change Thr300Ala is most commonly associated with the increased risk for developing CD (Patel & Stappenbeck, 2013). IRGM which is part of the p47 immunity-related guanosine triphosphatase family is also implicated in elimination of invasive pathogens (Palomino-Morales et al., 2009). Depletion of IRGM in human intestinal epithelial cells and macrophages has been linked to increased intracellular replications of AIEC and MAP (Nguyen et al., 2013).

**Other genetic loci**

Mutations in TLR4, leucine-rich repeat kinase 2 (LRRK2) and neutrophil cytosolic factor-4 (NCF4), have also been identified as CD susceptibility loci and are involved in bacterial recognition and phagocyte function (Tawfik et al., 2014). Similarly, defects in MUC19, IL23R and IL27 genes, which are primarily linked to mucus production to maintain integrity of the intestinal epithelial barrier and thereby protection against bacteria, have also been implicated in disease (Sheehan et al., 2015).

In conclusion, there is little doubt that bacteria contribute to CD pathogenesis. An alteration in the diversity of microbiota with advanced growth of invasive bacteria is documented in patients with CD. However, several key questions remain concerning the precise contribution of dysbiosis and the contribution of a single bacterial agent. Underlying triggers for dysbiosis are not well defined; however, exposure to antimicrobial agents and certain environmental factors, most notably dietary habits, appears to alter microbial diversity and limit host–microbe cross-talk. Collectively, the current microbial data indicate that altering the intestinal microbiome is critical in the disruption of mucosal homeostasis, which subsequently drives gut inflammation. For a few loci, CD pathogenesis can be suitably described by inappropriate interaction of mutant proteins and the microbiota. However, the precise contribution of genetics to the majority of CD remains unresolved. The heterogeneity of CD contributes to the complexity in defining the microbial contribution to CD pathogenesis, and it is likely that microbial contributions vary amongst CD patients. Better understanding of the microbiota–host interactions and how the microbiota community functions, therefore, seem crucial for new insights into microbial imbalances and how this imbalance contributes to pathogenesis in IBD.

**REFERENCES**


Dalton, J. P., Desmond, A., Shanahan, F. & Hill, C. (2014). Detection of *Mycobacterium avium* subspecies paratuberculosis in patients with Crohn’s disease is unrelated to the presence of single nucleotide polymorphisms rs2241880 (ATG16L1) and rs10045431 (IL12B).


The role of bacteria in Crohn disease


