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ACUTE ENTERIC INFECTIONS ALTER COMMENSAL MICROBIOTA: NEW MECHANISMS IN POST-INFECTIOUS INTESTINAL INFLAMMATORY DISORDERS

ANDRE G. BURET and KRISTEN RETI

Department of Biological Sciences, Inflammation Research Network, Host-Parasite Interactions Program, Snyder Institute for Chronic Diseases, University of Calgary, AB, Canada

SUMMARY

This chapter discusses how acute enteric infections may lead to post-infectious complications. Particular emphasis is given to infections with *Campylobacter jejuni* and *Giardia intestinalis*, two of the most common causes of enteric infections worldwide. The review provides a critical discussion of the biology of the human intestinal microbiota. Chronic post-infectious sequelae of these infections include malnutrition, stunting, failure to thrive, and impaired cognitive functions. They may also cause post-infectious disease at extra-intestinal sites, including the joints, the skin, the eyes, the lungs, the heart, the muscles, the kidneys, and the central nervous system. Findings from recent and ongoing research suggest that enteropathogen-induced disruptions of the commensal microbiota may at least in part play a role in triggering the sequence of events that result in these presentations. These disruptions include a promotion of the transcellular and paracellular translocation of non-invasive commensal bacteria, as well as disruptions of the composition and structure of microbiota biofilms.

INTRODUCTION

Diarrhoeal disease resulting from enteric infections remains one of the major causes of morbidity and mortality worldwide. Each year, an estimated 2 to 2.5 million children under the age of 5 years die from the 1.4 billion yearly diarrhoeal episodes in the paediatric population of developing countries (*O'Ryan* et al., 2005). Approximately 20 parasitic, viral, and bacterial pathogens are known to be the most common causative aetiologies (Table 1). Infection most commonly occurs through ingestion of contaminated food or water, or through direct faecal oral infection. Host factors, such as the host's nutritional and immune status, as well as environmental factors, like co-infections, are important in determining symptom severity. Indeed, some enteropathogens worsen the outcome of concurrent infections while others, like *Giardia intestinalis*, may partly protect children against diarrhoeal disease in developing countries (*Moore*, 2001; *Jensen* et al., 2009; *Veenemans* et al., 2012). Intestinal parasitic helminths are known to posses potent immune-regulating properties that may help attenuate tissue damage (*Maizels* et al., 2003), but overall, the mechanisms directing the clinical outcome of co-

Table 1: Enteropathogens most commonly reported as causes of acute diarrhoea worldwide. Most of these have been reported to have long-term sequelae via mechanisms that remain incompletely understood.

Parasites:	*Giardia intestinalis* (syn. *lamblia*, or *duodenalis*) *Cryptosporidium hominis* and *C. parvum* *Entamoeba histolytica*
Bacteria:	*Campylobacter jejuni* and *C. coli* Diarrhoeagenic *Escherichia coli* (ETEC- enterotoxigenic; EPEC - enteropathogenic; STEC - shiga-toxin producing; EAEC; enteroadherent; EIEC - enteroinvesive) *Salmonella* sp. *Shigella* sp. *Vibrio cholera* *Aeromonas* sp.
Viruses:	Rotavirus Norovirus Sapovirus Astrovirus Enteric adenovirus

infections and polyparasitism remain obscure. In developed countries of the World, public media warn that prevalence of enteric infections seems to increase. Indeed, the Wall Street Journal (3/15/2012, Martin) reported that according to CDC estimates, every year approximately 48 million Americans become ill through contaminated food, and 3,000 die. In its "National Briefing," the New York Times (3/15/2012, A20, Grady) reported that the CDC observes that "Deaths from gastrointestinal infections more than doubled in the United States from 1997 to 2007, to more than 17,000 a year from 7,000 a year.

Adding to the raising concerns caused by acute enteric infections, recent observations indicate that post-infectious complications may arise following exposure to a variety of enteropathogens, including *Campylobacter jejuni,* diarrhoeagenic *Escherichia coli, Salmonella* sp.*, Shigella* sp.*, Cryptosporidium parvum,* and *Giardia intestinalis*. In developing countries, acute diarrhoeal disease caused by these enteropathogens can lead to failure to thrive, stunted growth, and impaired cognitive functions. Recent studies following outbreaks of intestinal infections, and large retroactive cohort studies, also found that these infections may be responsible for chronic fatigue syndrome, arthritis, irritable bowel syndrome (IBS), and flare-ups in patients with Inflammatory Bowel diseases (IBD) (*Rodriguez* and *Ruigomez*, 1999; *Riddle* et al., 2001; *Berkman* et al., 2002; *Gradel* et al., 2009; *Kalischuk* and *Buret*, 2010; *Moore* et al., 2011; *Wensaas* et al., 2012). As a result, from being a leading cause of global child death, infectious diarrhoea now appears to have become a key source of lifelong morbidity (Table 2). The causes of the post-infectious clinical manifestations due to enteric infections, even after complete elimination of the enteropathogen, remain obscure. However, the commonality of these post-infectious disorders raises the intriguing possibility that they may share at least some of their basic biological mechanisms, hence offering great potential for the identification of novel therapeutic targets. This chapter elaborates on

Table 2: Examples of post-infectious complications and disorders reported following enteric infections, with *C. jejuni, E. coli, Salmonella* sp*., Shigella* sp*., Cryptosporidium parvum, G. intestinalis*, or viral enteropathogens.

Affected site:	Disorder/condition:	References:
Intestine:	Food allergies	Farthing et al., 1986; Hardin et al., 1997; Di Prisco et al., 1998
	Post-infectious irritable bowel syndrome	Rodriguez and Ruigomez, 1999; Spiller et al., 2000; Riddle et al., 2001;; Dizdar et al., 2007; Stark et al., 2007; Thabane et al., 2007; Gradel et al., 2009; Hanevik et al., ,2009; Marshall et al., 2010; Robertson et al., 2010; Wensaas et al., 2012; Buret et al., 2013; Simren et al., 2013
	Flare-ups in inflammatory bowel diseases	Kalischuk and Buret, 2010; Riddle et al., 2001; Swidsinsky et al., 2009; Reiff and Kelly, 2010; Buret et al., 2013
	Coeliac disease	Riddle et al., 2001
Joints:	Arthritis	Keat, 1991; Borman et al., 2001; Carlson and Finger, 2004; Schiellerup et al., 2008; Scher and Abramson, 2011
Skin:	Urticaria/Pruritus/Dermatitis	Di Prisco et al., 1998; Giacometti et al., 2003; Pietrzak et al., 2005
Eyes:	Iridocyclitis/Choroiditis/Retinal haemorrhages	Pettoelo-Mantovani et al., 1990; Corsi et al., 1998
Lungs:	Asthma,	Di Prisco et al., 1998; Han et al., 2012
	Obstructive lung disease	Hity et al., 2010
Heart:	Endocarditis	Miki et al., 2005
Muscles:	Hypokalaemic myopathy	Cervelló et al., 1993; Addiss and Lengerich, 1994; Genovese et al., 1996
CNS:	Impaired cognitive function	Guerrant et al., 1999; Berkman et al., 2002; Niehaus et al., 2002; Simsek et al., 2004; Koruk et al., 2010; Forsythe and Kunze, 2013; Bergman and Graham, 2005
	Autism	Mulle et al., 2013
	Guillain Barré Syndrome (paralysis)	Willison and O'Hanlon, 2000; Willison, 2005
Kidneys/ Urethra:	Haemolytic uremic syndrome	Delans et al., 1984
Entire body:	Stunting	Farthing et al., 1986; Guerrant et al., 1999; Berkman et al., 2002; Simsek et al., 2004; Ignatius et al., 2012
	Chronic Fatigue Syndrome	Mørch et al., 2009; Naess et al., 2012; Wensaas et al., 2012

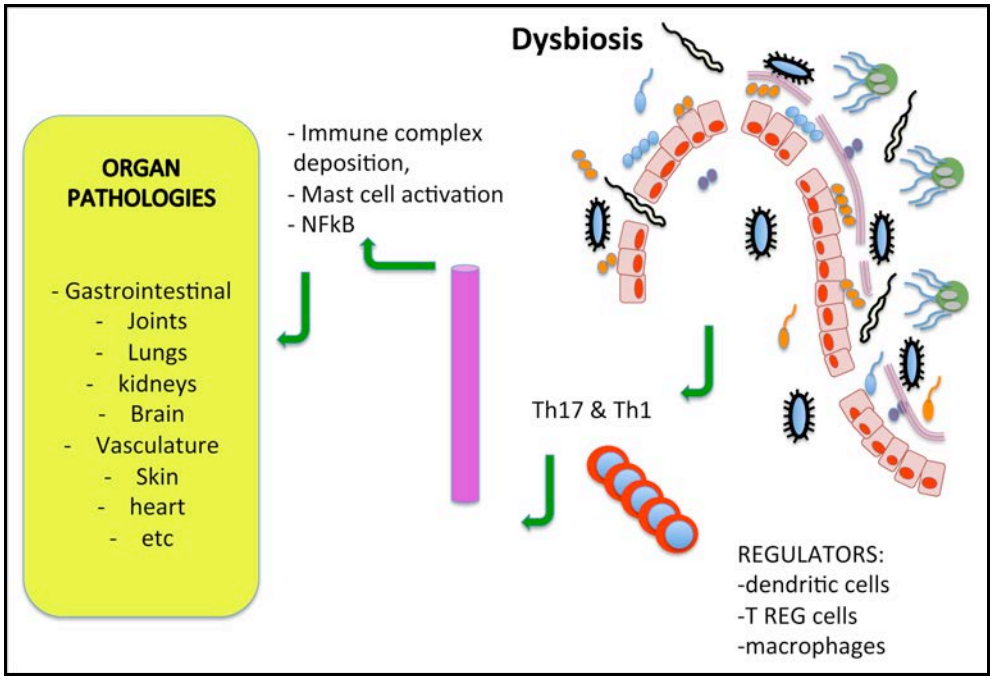

Figure 1: Hypothetical mechanisms, inferred from published research, by which acute enteric infections may lead to post-infectious, chronic complications. Enteropathogens, including *Campylobacter jejuni* or *Giardia intestinalis* cause microbiota biofilm dysbiosis, and disruptions of gut homeostasis and barrier function (*Kalischuk* and *Buret*, 2010; *Cotton* et al., 2011; *Buret* et al., 2013). These effects may be further compounded by a direct breakdown of the mucus barrier via mucin degradation by the enteropathogen (*Macfarlane* et al., 1999, 2005; *Moncada* et al., 2005; *Derrien* et al., 2010). In turn this causes proliferation of autoreactive cells like Th17 and Th1 lymphocytes, and release of pro- inflammatory cytokines (including IL-17, IFN-γ, TNF, etc.). Local dendritic cells, macrophages, and T Regulatory cells play a central role in regulating these processes. As the effects reach the circulation, this leads to immune complex deposition, tissue mast cell degranulation, NF-κB activation, and/or other yet unknown processes that cause organ pathology and disease (*Hardin* et al., 1997; *Hity* et al., 2010; *Philpott* et al., 2011; *Scher* and *Abramson*, 2011; *Han* et al., 2012; *Forsythe* and *Kunze*, 2013; *Mulle* et al., 2013).

recent findings suggesting that acute enteritis may disrupt the intestinal homeostatic balance between the intestine of the host and its own microbiota, and that these alterations in turn may be responsible for initiating the sequence of events culminating in post-infectious symptomatology. Because *Campylobacter jejuni* and *Giardia intestinalis* are some of the most common causes of bacterial and parasitic enteritis worldwide, the chapter will emphasize on findings using these two disease models primarily (*Savioli* et al., 2006; WHO: Campylobacter. Fact sheet N°255, October 2011 http://www.who.int/mediacentre/factsheets/fs255/en/).

BIOLOGY OF THE INTESTINAL MICROBIOTA

Intestinal microbiota and host have evolved to live in tolerant commensalism. Indeed, host immunity and gut physiology are shaped by the microbiota, which itself is modulated by host immune, genetic, dietary and other

environmental factors. Recent evidence also indicates that disruptions of these microbiota influences homeostasis to cause disease at extra-intestinal sites, including asthma, obstructive lung disease, arthritis, and even disorders of the central nervous system such as autism (*Hity* et al., 2010; *Scher* and *Abramson*, 2011; *Han* et al., 2012; *Forsythe* and *Kunze*, 2013; *Mulle* et al., 2013). Much of the findings remain association-based, and cause-to-effect relationship studies are now sorely needed. Over 70% of the gut microbiota have not yet been cultured or classified, but new culture-independent techniques have established that these microbial communities are host- and GI tract location-specific, and that they play a key role in health (*Zoetendal* et al., 2008; *de Weerth* et al., 2013; *Lepage* et al., 2013). Indeed, recent advances in sequencing, metagenomics, and bioinformatics technology have found that the estimated 10^{14} human gut microorganisms, weighing a rough total of 3 pounds, contain an overall genome size 150 times larger than that of the human genome (*Zoetendal* et al., 2008; *de Weerth* et al., 2013; *Lepage* et al., 2013). Moreover, these studies found that gene exchanges between representatives of the gut microbiota were in fact much more common than previously anticipated (*Zoetendal* et al., 2008). This raises the question as to whether or not such exchanges may also be common between enteropathogens and the normal microbiota. In the early stages of life, the gut microbiota undergoes some degree of shifting, but overall, in the later parts of life, it remains stable in the absence of major disturbances of the host's health conditions and diet (*Dethlefsen* and *Relman*, 2011). To date, the phylogenetic core of the human microbiota is thought to be composed of 60-70 highly prevalent species (*Tap* et al., 2009). Loss of diversity of the microbiota, which may occur via mechanisms that remain unclear, has been associated with disease (*Frank* et al., 2007). Taken together, these new insights from DNA sequence-based analyses of gut microbial communities suggest that the microbiome represents a key environmental factor that can influence disease manifestation (Figure 1). How in turn acute enteric infections may be at the source of pathogenic microbiota disruptions has become a very intriguing part of this puzzle.

The population of industrialized countries, with their characteristic high fat, high protein diets, harbour different microbiota than those living in rural areas of developing countries, with a polysaccharide-rich diet (*De Filippo* et al., 2010)). The differences mainly reflect an increased representation of *Bacteroidetes* in the latter group, a group of bacteria known for its high genetic ability to hydrolyse xyloses. The relative sensitivity of these distinct microbiota to enteropathogens, and how in turn disruptions in their respective flora may differentially regulate post-infectious disorders, is unknown.

Microbial communities colonizing the gut do so in a gradient, from few organisms in the oesophagus and stomach, to the much more heavily colonized colon (*Hokins* et al., 2002). Ever since the late 19th century, when Robert Koch's studies in Germany developed the germ theory of disease, bacteria were envisioned as single cells that float or swim through some kind of watery habitat, within the human body, or in the environment in which they lived. With the giant progresses made in microbiology since then, and in the large part only since the 1960's,we now understand that the swimming bacteria in typical laboratory cultures act nothing like the ones encountered in nature. Indeed, many of these organisms do not,

in the real world, spend much time swimming freely as isolated cells. Instead, they stick to wetted surfaces in organized colonies that form a complex multi-species slime-enclosed film, allowing them to withstand environmental flushing forces, as well as resist against antimicrobials and competing microbes. These slime-producing communities are known as bacterial "biofilms" (*Hall-Stoodley* et al., 2004). The slimy coating in which they live contains more than 90% water and complex microbially secreted exo-polysaccharides that confer viscosity. The GI tract, with its constant luminal flow and high nutrient availability make it an ideal site for bacteria to live as biofilms. While we now understand that gut microbiota do indeed live in a biofilm phenotype, much remains to be learnt on how the integrity and physiology of these communities may bear on gut homeostasis and disease (*Von Rosenvinge* et al., 2013). Importantly however, as these biofilms live in very close proximity to host epithelial cells, they are the microbial communities most likely to interact with host physiology, immunity, and metabolism. As such, more so than studies on faecal microbiota, research now needs to investigate the bacterial biofilms living on the mucosal surface of the intestine, as it is likely to shed key elements of our understanding of host-gut microbiota interactions. Indeed, biofilm microcolonies exist on the intestinal mucosal surface in healthy people, and these bacterial populations are different from those living in the intestinal lumen (*Macfarlane* and *Macfarlane*, 1992). The layer of mucus coating the epithelial surfaces of the gut prevents most microorganisms from reaching and/or persisting on the mucosal surface. However, mucus glycoproteins, including mucins, represent an important source of carbohydrates for the saccharolytic biofilm bacteria, particularly at site where fermentable carbohydrates may be in small supply, such as in the colon (*Macfarlane* et al., 1992). This breakdown of mucin appears to be a cooperative undertaking by the microbiota, meant to benefit the entire bacterial biofilm community (*Macfarlane* and *Macfarlane*, 1992). Intriguingly, intestinal biofilm bacteria living on mucin differ metabolically and phylogenetically from those living in a planktonic state (*Macfarlane* et al., 2005). Together, these data demonstrate the physiological significance of the biofilm mode of life of the intestinal microflora. A number of reports have now established that patients with IBD and IBS have a reduced microflora diversity, and an apparent reduction in the commensal phyla *Firmicutes* and *Bacteroidetes*, as well as increased numbers of *Proteobacteria* (*Reiff* and *Kelly*, 2010; *Simren* et al., 2013). Furthermore, recent evidence from fluorescent *in situ* hybridization labelling (FISH) indicates that mucosal bacterial colonies live in closer proximity to the epithelial lining in IBD than in normal individuals (*Swidsinsky* et al., 2009). Whether this results from a more "adherent-invasive" phenotype in the bacteria found in patients with IBD requires further clarification. Indeed, involvement of the commensal microflora in the initiation, persistence, and flare-ups of IBD has been suggested as early as 1971 (*Hill* et al., 1971). However, much remains to be learnt about the role of microbiota biofilm disruptions in the aetiology of post-infectious complications following acute enteric infections. Moreover, research is needed to assess the effects of enteropathogens on gut biofilm integrity, in an attempt to identify new mechanisms that lead to the severe intestinal and extra-intestinal post-infectious complications of acute enteric infections.

CAMPYLOBACTER JEJUNI

Campylobacter jejuni is one of the most prevalent causes of human bacterial enteritis in the World (*Moore*, 2001; *O'Ryan* et al., 2005; Public Health Agency of Canada, URL: http://www.phac-aspc.gc.ca/id-mi/az-index-eng.php). About 90% of human campylobacteriosis is caused by *C. jejuni*, and the remaining 10% is induced predominantly by *C. coli.* In the paediatric population of developing countries however, diarrhoeagenic *E. coli* (EPEC, ETEC, STEC, EAEC, and EIEC) remain the most common bacteria detected during diarrhoea, and these bacteria represent 30-40% of acute diarrhoeal episodes in children (*O'Ryan* et al., 2005). Though polymicrobial infections involving *Campylobacter* appear to be less common in developed countries, the bacterium is frequently isolated with other enteropathogens in the developing World; co-infecting organisms include *Escherichia coli*, *Salmonella sp.*, *Shigella sp.*, *Giardia intestinalis*, and *rotavirus* (*Coker* et al., 2002; *Janssen* et al., 2008).

Inadequate hygiene represents a significant contributor to campylobacteriosis, as is the case for infections with *Shigella* sp.*, Salmonella* sp., and other enteropathogens. *C. jejuni* lives as a non-pathogenic enteric bacterium in poultry and cattle. Upon ingestion of contaminated water or food (e.g. raw milk), infected humans exhibit a range of symptoms varying from mild to severe diarrhoea. Clinically therefore, acute campylobacteriosis is not readily distinguishable from other enteric infections. These clinical features typically arise 2-4 days post-infection and are indicative of the inflammatory response that is occurring (Public Health Agency of Canada, URL: http://www.phac-aspc.gc.ca/id-mi/az-index-eng.php). Histological examination of affected intestinal tissues reveals a broad spectrum of tissue alterations, and, most commonly, infiltration of neutrophils into the lamina propria secondary to NF-κB activation (*Wassenaar* et al., 1999). The disease seems to be less severe in developing countries (*Coker* et al., 2002).

Pathogenesis

The host-pathogen interactions responsible for inciting inflammation remain incompletely understood (*Janssen* et al., 2008). Tissue damage appears to be largely due to the effects of cytotoxins, and/or host-cell invasion (*Wassenaar* et al., 1999; *Russell* et al., 1993). Recent findings suggest that a *C. jejuni* lipoprotein (JlpA) promotes epithelial adhesion, and the subsequent induction of pro-inflammatory signalling (*Jin* et al., 2003). Moreover, *C. jejuni* produces cytolethal distending toxin (Cdt), a DNase-like toxin produced by several species of bacteria. This holotoxin is composed of three subunits: CdtB belongs to the family of DNase I-like nucleases and is the active subunit, while the CdtA and C subunits help deliver CdtB into target cells (*Bang* et al., 2001). Upon epithelial invasion by *C. jejuni*, Cdt translocate into the nucleus, where it activates the G1 and/or G2/M checkpoint responses, resulting in cell cycle arrest, and ultimately cell death via mechanisms that are poorly understood (*Whitehouse* et al., 1998; *Bang* et al., 2001). The effects of Cdt have primarily been described for lymphocytes and monocytes, in which it appears to induce apoptosis (*Hickey* et al., 2005). However, Cdt has also been shown recently to induce non-apoptotic death of endothelial cells (*Bielaszewska* et al., 2005). In addition, *C. jejuni* is thought to produce several other cytotoxins besides Cdt, including shiga-like toxin,

haemolysin, and hepatotoxin (*Wassenaar* et al., 1997). These cytotoxic factors require further characterization, and their possible implication in *C. jejuni*-induced epithelial injury is poorly understood. *C. jejuni* flagella represent another important virulence factor, as they promote motility through the mucus layer, and are important in the adherence to and invasion of epithelial cells (*Wassenaar* et al., 1991; *Konkel* et al., 2004; *Christensen* et al., 2009). Interestingly, strain-dependent induction of epithelial cell oncosis by *C. jejuni* correlates with invasion ability, but may occur independently of Cdt (*Kalischuk* et al., 2007). Regardless, the acute stage of the infection is able to break the intestinal barrier, which in turn allows luminal antigens to stimulate subepithelial immunity. In the acute stage of infection, *C. jejuni* also disrupts protective TLR-9 signalling in epithelial cells (*O'Hara* et al., 2012). These alterations in turn prime the intestine for heightened inflammatory injury upon mild pro-inflammatory stimulation, via mechanisms that remain incompletely understood (*O'Hara* et al., 2012).

Microbiota disruptions by Campylobacter sp.

C. jejuni facilitates the translocation of non-invasive, commensal bacteria, both paracellularly as well as transcellularly. The latter occurs by hijacking the host lipid raft pathway as well as via epithelial M cells (*Lamb-Rosteski* et al., 2008; *Kalischuk* et al., 2009, 2010). On-going research has also uncovered that *C. jejuni* is able to directly disrupt the composition and integrity of human microbiota biofilms, which may lead to a loss of tolerance by the host to its own commensal microbiota (*Buret* et al., 2013). Interestingly, the effects of *C. jejuni* on microbiota biofilms can be duplicated with *G. intestinalis*, but not with a commensal *E. coli* (*Buret* et al., 2013). Further research is warranted to clarify the sequence of events responsible for the post-infectious complications of campylobacteriosis, which will help unravel common pathways through which acute enteropathogens cause post-infectious inflammatory disorders.

Post infectious complications

Long after the *C. jejuni* infection has been cleared, some individuals experience abnormal bowel patterns (more frequent, watery stools; or fewer hard/lumpy stools) that may persist for years (*Riddle* et al., 2001; *Marshall* et al., 2010). These abnormalities may be associated with intestinal enteroendocrine cell hyperplasia, and low-grade inflammation, including an increase in CD3 lymphocytes and proliferation of intraepithelial lymphocytes (*Spiller* et al., 2000).

The post-infectious complications caused by acute campylobacteriosis include Guillain-Barré syndrome, reactive arthritis, Reiter syndrome (an inflammatory disease with either conjunctival or urethral inflammation), Irritable Bowel Syndrome, flare-ups in patients with Inflammatory Bowel Diseases, and possibly celiac disease (*Keat* and *Rowe*, 1991; *Riddle* et al., 2001; *Coker* et al., 2002; *Janssen* et al., 2008; *Marshall* et al., 2010). On rare occasions, *C. jejuni* infection may also cause haemolytic-uremic syndrome, a well-known consequence of infection with enterotoxigenic *Escherichia coli* (*Delans* et al., 1984). Another rare extra-intestinal complication of campylobacteriosis is endocarditis (*Miki* et al., 2005). Intriguingly, the symptoms of post-infectious arthritis appear to be similar regardless of the infecting bacterial species, indicating a role for factors common to a range of pathogens (*Schiellerup* et al., 2008). Guillain-Barré Syndrome may develop in ap-

proximately 1 in 1,000 infected individuals infected with *C. jejuni*, and is a serious autoimmune neurological disorder; symptoms may range from weakness of extremities to complete paralysis and respiratory insufficiency (*Willison*, 2005). The majority of patients may recover completely within 6 to 12 months (*Willison*, 2005). Guillain-Barré Syndrome is thought to occur because of molecular mimicry between the lipo-oligosaccharide of the *C. jejuni* cell envelope, and sugar moieties on nerve gangliosides (*Willison* and *O'Hanlon*, 2000). In turn, antibodies raised during infection with *C. jejuni* may cross-react with nerve gangliosides in some individuals, leading to the demyelinization of nerves, and subsequent degeneration of axons (*Willison*, 2005).

Irritable bowel syndrome (IBS), is the most commonly diagnosed functional gastrointestinal disorder by gastroenterologists, and is characterized by abdominal hypersensitivity and abnormal bowel movement (diarrhoea and/or constipation). It is a common long-term consequence of acute gastroenteritis caused by a variety of enteropathogens, including *C. jejuni*, *Samonella* sp., diarroeagenic *E. coli*, and *Giardia intestinalis* (*Riddle* et al., 2001; *Thabane* et al., 2007; *Ohman* et al., 2010). Altered motility patterns as well as abdominal pain in post-infectious IBS have been associated with mast cell secretions such as mast cell tryptase and serotonin (5-hydrocytryptamin, 5-HT), also released from enterochromaffin cells (*Cenac* et al., 2007; *Cremon* et al., 2011).

Finally, it was recently demonstrated that acute gastroenteritis with *C. jejuni*, diarrhoeagenic *E. coli*, or *Salmonella* sp. may lead to the initiation and or exacerbation of Inflammatory Bowel Diseases (IBD; Crohn's Disease and ulcerative colitis), themselves associated with rheumatic manifestations, further linking gut disturbance to osteoarticular disorders (*Gradel* et al., 2009; *Rodriguez-Reyna* et al., 2009). The mechanisms remain unclear. Several inflammatory factors implicated in IBD implicate the NF-κB pathway. In keeping with a prominent role for microbes in the pathogenesis of IBD, a variety of bacterial products, including bacterial lipopolysaccharide (LPS), are also potent activators of this pathway. Polymorphic mutations of NOD2 (also called CARD15), which acts as an intracellular sensor of bacteria-derived muramyl dipeptide (a component of Gram-positive and Gram-negative bacterial cells walls) are the product of the IBD1 gene mutation present in some patients with IBD, and significantly increase disease susceptibility by altering the NF-κB pathway (*Ahmad* et al., 2002; *Podolsky*, 2002). The cytosolic NOD2 receptor may also activate the NF-κB pathway upon exposure to LPS which may have entered the cytoplasm via mechanisms that have yet to be elucidated (*Ahmad* et al., 2002; *Podolsky*, 2002). The increased numbers of *E. coli* and *Proteobacteria* detected in the intestinal mucosa of IBD patients may heighten exposure to pathogenic products such as lipoproteins, proteoglycans, and LPS. Other components of the resident microflora, within the stressed and inflamed environment of the IBD intestine, may also activate host inflammation via mechanisms that are incompletely understood (*MacPherson* et al., 2004). In addition, upregulated expression and/or polymorphic mutations of receptors for LPS, e.g. TLR-4, have been found in epithelial cells of IBD patients (*Cario* et al., 2000). LPS has the ability to break the intestinal barrier (*Qi* et al., 2005; *Yu* et al., 2005; *Chin* et al., 2006). It has also been recently reported that peripheral blood monocytes from IBD patients

exhibit increased TLR2 expression, and this is correlated with a marked increase of TLR-2 -mediated TNF-α production (*Cantó* et al., 2006). The notion that circulating LPS and anti-endotoxin antibodies can be found in the plasma of IBD patients is consistent with a breach in the epithelial barrier (*Gardiner* et al., 1995). Activation of TLR-4 appears to be implicated in the development of pathology during infectious colitis (*McKay*, 1999; *Cario* et al., 2000). Interestingly, a recent study also demonstrated that *C. jejuni* infection disrupts TLR-9 signalling, which makes the intestinal mucosa more prone to inflammatory injury (*O'Hara* et al., 2012). Furthermore, *in situ* examination of biopsies from patients with IBD revealed the increased uptake of non-invasive, commensal *E. coli* via the follicle-associated epithelial M cells, a phenomenon known to be facilitated by *C. jejuni* (*Keita* et al., 2008). These invading commensal *E. coli* were shown to co-localize with dendritic cells, which correlated with increased levels of the pro-inflammatory cytokine TNF-α. Disruptions of the intestinal barrier by enteropathogens may permit luminal material, including commensal bacteria and/or their products, to activate baso-lateral pro-inflammatory sensors like TLR's which otherwise may have been inaccessible. Therefore, luminal factors capable of breaching epithelial integrity, and/or altering the polar distribution of TLR's, may predispose the intestine to heightened intestinal inflammation in a susceptible host. Findings from on-going research indicate that *C. jejuni*- or *Giardia*-induced disruptions of the microbiota biofilm composition and integrity may help trigger a sequence of events that may lead to post-infectious complications (*Buret* et al., 2013). A better understanding of the disruptions to the resident microflora and deregulated bacterial recognition secondary to acute *C. jejuni* infection may shed new light on the mechanisms responsible for the initiation and/or exacerbation of inflammation in IBD patients.

Taken together, findings have started to establish processes through which campylobacteriosis may lead to post-infectious sequelea. These include inflammatory disorders in the gut, but may also affect extra-intestinal sites, including the central nervous system, the lungs, the kidneys, the eyes, the joints, and even the heart. This characteristic is shared with the post-infectious complications caused by a variety of other enteropathogens, further supporting the hypothesis of common pathogenic pathways. At least part of these processes appear to be triggered by enteropathogen-induced disruptions of the host microbiota biofilms. More research needs to identify the mechanisms through which *Campylobacter*, and other enteropathogens, may trigger events in the microbiota and the intestinal mucosa that ultimately set the stage for chronic inflammatory disorders in the gut, as well as at extra-intestinal sites.

GIARDIA INTESTINALIS

Giardiasis, caused by *G. intestinalis* (synonymous *G. lamblia* or *G. duodenalis)*, is the most common waterborne parasitic infection of the human intestine worldwide, and was recently included in the World Health Organisation's Neglected Disease Initiative (*Savioli* et al., 2006; WHO: Guidelines for drinking water quality, 3rd Edition http://www.who.int/water_sanitation_health/dwq/gdwq3/en/). The prevalence of human giardiasis is highest in devel-

oping countries, where it ranges from 20% to 100% of the population, versus its prevalence of 3-7% in developed countries (*Jensen* et al., 2009; *Ankarlev* et al., 2010). People infected with *Giardia* may develop a broad range of clinical manifestations, ranging from asymptomatic infection, to acute or chronic diarrhoeal disease associated with abdominal pain and nausea (*De Filippo* et al., 2010; *Cotton* et al., 2011). Most infections are self-limiting, although re-infection and chronic infection can occur. Recent evidence indicates that *G. intestinalis*, like *C. jejuni* and other enteropathogens, is responsible for chronic post-infectious complications, via mechanisms that remain obscure.

Pathogenesis

Pathophysiology in giardiasis occurs without invasion of the small intestinal tissues by the trophozoites, and in the absence of any overt inflammatory cell infiltration, with the exception of a modest increase in intraepithelial lymphocytes; some of the acute pathology, which involves a diffuse shortening of epithelial microvilli, is caused by activated CD8+ lymphocytes (*Buret* et al., 1992; *Scott* et al., 2004). As is the case for enteric infections caused by *Campylobacter* sp., diarrhoeagenic *E. coli*, *Salmonella* sp., and others, the pathophysiology of acute diarrhoea in giardiasis implicates a disruption of the intestinal barrier function. In giardiasis, heightened rates of enterocytes apoptosis, intestinal barrier dysfunction, activation of host lymphocytes, shortening of brush border microvilli with or without coinciding villous atrophy, disaccharidase deficiencies, small intestinal malabsorption, anion hypersecretion and increased intestinal transit rates all seem to contribute to disease (*Buret* et al., 1992; *Chin* et al., 2002; *Scott* et al., 2004; *Troeger* et al., 2007; *Koot* et al., 2009; *Cotton* et al., 2011). Whether these effects may be further compounded by degradation of local mucins by *Giardia*, as it was found for other enteric microbes like *E. histoloytica*, requires further investigation (*Macfarlane* and *Macfarlane*, 1999; *Macfarlane* et al., 2005; *Moncada* et al., 2005; *Derrien* et al., 2010). As is the case with other enteropathogens, induction of apoptosis in enterocytes by *Giardia* represents a key component in the pathogenesis of the infection (*Chin* et al., 2002; *Panaro* et al., 2007; *Troeger* et al., 2007; *Buret* et al., 2013). The mechanisms responsible are unknown, and the identification of a *Giardia* "enterotoxin" has remained elusive. *Giardia*-mediated increases in intestinal permeability result from alterations to the apical junctional complexes, including disruptions of F-actin, zonula-occludens (ZO)-1, claudin-1, and α-actinin, a component of the actomyosin ring that regulates paracellular flow, under the control of epithelial myosin light chain kinase (*Teoh* et al., 2000; *Scott* et al., 2002; *Cotton* et al., 2011; *Maia-Brigagão* et al., 2012). The mechanisms leading to loss of intestinal barrier function caused by *Giardia* sp. are shared among a broad range of enteropathogens (*O'Hara* and *Buret*, 2008).

Microbiota disruptions by Giardia

Bacterial components of the microbiota from patients with symptomatic giardiasis appear to heighten *G. intestinalis* virulence in gnotobiotic mice, via unclear mechanisms (*Torres* et al., 2000). Little is known of the effects of acute giardiasis on the human commensal microbiota. But findings from on-going studies indicate that indeed, *G. intestinalis* is able to disrupt the composition and integrity of human intestinal microbiota biofilms, in a fashion similar to what *C. jejuni* does (*Buret* et al.,

2013). More research in this area will help identify common pathways through which acute enteritis may lead to long term inflammatory disorders by disrupting commensal bacterial biofilms, as well as how these may lead to extra-intestinal complications.

Post-infectious complications of giardiasis

Infection with *Giardia* sp. may lead to food allergies, negatively affect nutritional and growth status, and impair cognitive function in humans (*Farthing* et al., 1986; *Berkman* et al., 2002; *Niehaus* et al., 2002; *Ettehad* et al., 2010; *Ignatius* et al., 2012). Recent evidence also indicates that 5-10% of patients diagnosed with giardiasis will develop post-infectious irritable syndrome and functional dyspepsia, long after clearance of the parasite (*Dizdar* et al., 2007; *Stark* et al., 2007; *Hanevik* et al., 2009; *Robertson* et al., 2010). When the infection persists for months, microscopic duodenal inflammation may develop (*Hanevik* et al., 2009; *Mørch* et al., 2009), further underscoring the need for rapid parasitic elimination to reduce the risk of chronic complications in giardiasis. Infection with *Giardia* can cause iron deficiency anaemia, micronutrient deficiencies, protein-energy malnutrition, which all have been linked to growth and cognitive retardation (*Simsek* et al., 2004; *Koruk* et al., 2010). Studies conducted in Brazil and Peru found that diarrhoeal disease occurring in the first 2 years of life negatively correlates with cognitive function, verbal fluency, and physical fitness, and may lead to long-term growth faltering (*Guerrant* et al., 1999; *Berkman* et al., 2002). Long-term sequelae of wasting and/or stunting often include general behavioural and developmental consequences that present as failure to thrive, which has also been linked to giardiasis (*Berkman* et al., 2002; *Bergman* and *Graham*, 2005; *Ettehad* et al., 2010). The persistence of infection and its association with diarrhoea are key factors associated with growth disturbance and failure to thrive, and diarrhoea caused by enteric infections in early childhood has become a predictor of stunting (*Berkman* et al., 2002; *Botero-Garcés* et al., 2009; *Ettehad* et al., 2010). In giardiasis and cryptosporidiosis, as well as other enteric infections, diarrhoea may lead to poor cognitive function by causing zinc and iron micronutrient deficiencies, as well as defects in the anti-oxidant system, which may all affect neuroplasticity (*Ajjampur* et al., 2011). Moreover, diarrhoea during early childhood was also found to impair visual-motor coordination, auditory short-term memory, information processing, and cortical cognitive function (*Guerrant* et al., 1999; *Ajjampur* et al., 2011). Combined with these complications, some individuals may develop post-giardiasis fatigue and musculoskeletal pain (*Naess* et al., 2012). Viral, bacterial, as well as parasitic pathogens have the ability to cause chronic fatigue syndrome. Recent studies have reported a high prevalence of post-infectious fatigue following a giardiasis outbreak in Bergen, Norway, in 2004 (*Dizdar* et al., 2007; *Hanevik* et al., 2009; *Mørch* et al., 2009; *Robertson* et al., 2010; *Naess* et al., 2012; *Wensaas* et al., 2012).

Beyond its long-term consequences on intestinal and overall metabolic parameters, giardiasis, like campylobacteriosis, and other enteric infections, also has the ability to cause post-infectious complications at extra-intestinal sites (*Cantey* et al., 2011). Sites affected include the eyes (*Pettoelo-Mantovani* et al., 1990; *Corsi* et al., 1998), the joints (*Borman* et al., 2001; *Carlson* and *Finger*, 2004), the skin (*Hardin* et al., 1997; *Di Prisco* et al., 1998; *Giacometti* et al., 2003; *Pietrzak* et al., 2005),

and on rare occasions, the muscles (*Cervelló* et al., 1993; *Addiss* and *Lengerich*, 1994; *Genovese* et al., 1996). These patterns of chronic post-infectious consequences again are similar to what has been reported for other enteric infections (Table 2).

CONCLUSIONS

Recent findings clearly demonstrate that the health consequences of enteric infections go far beyond their acute diarrhoeal symptoms, as they can lead to severe chronic post-infectious intestinal inflammatory disorders, failure to thrive, and serious growth and cognitive impairment. Moreover, the chronic sequelae may also cause post-infectious disease at extra-intestinal sites, including the joints, the skin, the eyes, the lungs, the heart, the muscles, the kidneys, and the central nervous system. The mechanisms responsible for these long-term effects remain obscure. However, findings from recent and ongoing research suggest that enteropathogen-induced disruptions of the commensal microbiota may at least in part play a role in triggering the sequence of events that result in these presentations. These disruptions include a promotion of the transcellular and paracellular translocation of non-invasive commensal bacteria, as well as disruptions of the composition and structure of microbiota biofilms. These in turn contribute, at least in part, to the activation of the host autoimmune reactions that are implicated in the production of post-infectious complications.

Beyond the well-established need to better understand host-pathogen cross-talks, as well as interactions between the host and its microbiota, these observations lay the foundations for future research into enteropathogen-microbiota interactions. This research will help better understand gut homeostasis, and will help unravel new pathophysiological pathways.

The direct benefits of microbiota are not well understood mechanistically. Most information is derived from the use of probiotics, and more recently the use of faecal microbiota transplant (FMT). With the recent decision of the Federal Drug Administration (U.S.A.) to call FMT a “drug” and hence to only allow its use under an Investigational New Drug application, (with a recently implemented exception for the treatment of *Clostridium difficile* infection), mechanistic insights into the now well-established beneficial effects of FMT may take a long time to become clear. This further underscores the need to uncover model systems that will allow to develop well-characterized, safe “synthetic microbiota” of “laboratory prepared” FMT-like cocktails.

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