We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS

BOOK CITATION INDEX

INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Clostridium difficile-Associated Colitis: Role of the Immune Response and Approach to Treatment

Katie Solomon[1] and Lorraine Kyne[2,3]
[1]School of Public Health, Physiotherapy and Population Science,
University College Dublin,
[2]School of Medicine and Health Sciences,
University College Dublin,
[3]Mater Misericordiae University Hospital, Dublin,
Ireland

1. Introduction

Clostridium difficile infection (CDI) is the most common infectious cause of healthcare-acquired diarrhoea. Approximately 15%–25% of all cases of antibiotic-associated colitis (AAC) are caused by *C. difficile* and this likelihood increases with the severity of disease, reaching 95%–100% among patients with documented antibiotic-associated pseudomembraneous colitis (PMC) (Bartlett, 1994). Since the initial report of *C. difficile* as the cause of AAC in 1978 (Larson *et al.*, 1978), subsequent work has provided important information regarding risk factors, diagnosis and effective therapy. More recently, significant challenges have arisen due to increases in frequency and severity of disease, limitations of standard therapy and propensity for recurrence of infection. There has been an unanticipated increase in morbidity and mortality attributed to this disease, linked in part to the emergence of antimicrobial-resistance and the epidemic bacterial strain, BI/NAP-1/ribotype 027 leading to a resurgence of CDI as a major cause of hospital-acquired infection.

2. Clinical presentation of CDI

Infection with *C. difficile* can result in clinical manifestations ranging from asymptomatic carriage to fulminant colitis and death (Bartlett, 1994). The pathogenesis of symptomatic CDI is characterised by an acute intestinal inflammatory response, prominent neutrophil infiltration and associated tissue injury (Savidge *et al.*, 2003). Features of severe CDI include pseudomembranes visible on endoscopy, abdominal cramps, fever, marked increase in white cell count, rise in serum creatinine level and hypoalbuminemia (Cloud *et al.*, 2009; Pepin *et al.*, 2009). Systemic symptoms may be absent in mild disease but are common in moderate or severe disease. Accurate diagnosis early in the disease course is important for the successful management of CDI.

2.2 Elevated white cell count and hypoalbuminemia

The increase in white cell count associated with CDI is mainly a result of a marked increase in peripheral blood neutrophils. The intense intestinal inflammatory response in severe CDI may also result in fever, abdominal pain, thickening of the colon and paralytic ileus that can evolve into toxic megacolon. Profuse watery diarrhoea may also be associated with nausea, vomiting, dehydration or lethargy, in addition to severe hypoalbuminemia (serum albumin <35g/L) as a result of protein losing enteropathy (Sunenshine & McDonald, 2006).

2.1 Pseudomembraneous colitis

Pseudomembranes are characterised as discrete plaques of yellow-white exudate <10mm diameter separated by normal or mildly hyperaemic mucosa usually restricted to the colon, with occasional cases involving the small intestine (Figure 1A &B). These may progress to form a membraneous exudate covering a large degree of the colonic epithelial surface. Ulceration into the sub-mucosa can result in severe cases. The lesions are characterised histologically as discrete regions of necrotic surface epithelium with accumulation of fibrin, mucous and cell debris (Pothoulakis, 1996). Polymorphonuclear cells and neutrophils infiltrate into the lesion exudate and the underlying lamina propria from the systemic bloodstream (Kelly *et al.*, 1994a). Later stage lesions involve the superficial mucosa and crypts and further infiltration of polymorphonuclear cells and eosinophils. Lesions coalesce to create plaques covering larger areas of the mucosa. Rare complications involve deep necrosis and ulceration of the colon sub-mucosa which can lead to perforation, septicaemia and death.
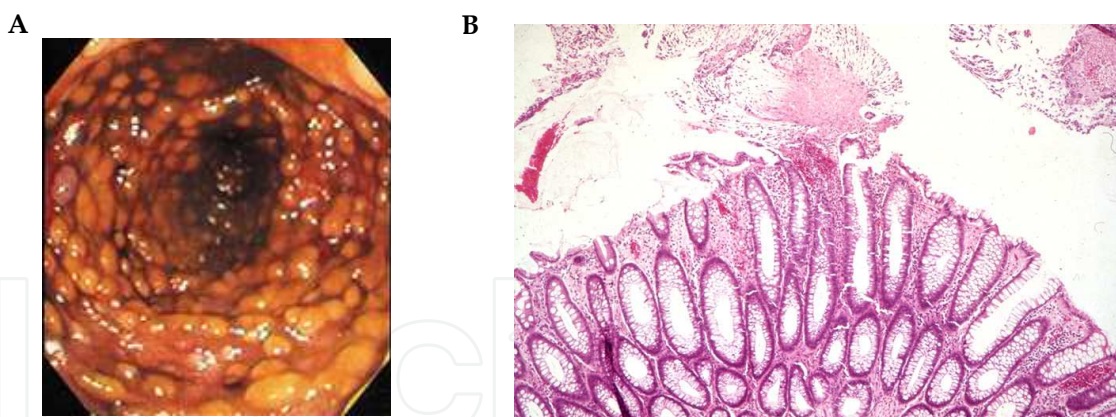

Fig. 1. (A) Pseudomembraneous colitis as confirmed by sigmoidoscopy. Discrete yellow plaques indicate the ulcerated lesions. (B) Histopathological features of pseudomembraneous colitis include loss of crypt structure, infiltration of polymorphonuclear cells and surface accumulation of fibrin, mucous and necrotic cell debris.

3. Risk factors

The major risk factors for CDI are increasing age, prolonged hospital stay and recent or current antimicrobial use. The most important risk factor is alteration of bowel microflora and subsequent loss of colonisation resistance associated with antimicrobial usage within

the previous 2-3 months prior to infection (Bignardi, 1998; Dial *et al.*, 2008). There are particular classes of antimicrobials that are associated with the highest risk of *C. difficile* acquisition, including clindamycin, cephalosporins and β-lactam antimicrobials and more recently fluoroquinolones (Owens *et al.*, 2008). Recent outbreaks involving a particular epidemic strain of *C. difficile* have been predominantly associated with fluoroquinolone usage (Loo *et al.*, 2005; Pepin *et al.*, 2005). The risk of developing CDI increases with the use of multiple antibiotics and prolonged duration of therapy.

In addition to these traditional risk factors, other factors associated with CDI include; underlying comorbidities, including inflammatory bowel disease (IBD), gastrointestinal procedures and exposure to an environment contaminated with toxigenic *C. difficile*, usually via hospitalisation (Johnson & Gerding, 1998). Proton pump inhibitors have been associated with CDI risk but their use is often a marker of the severity of underlying disease, which is in itself a strong risk factor for CDI (Kyne *et al.*, 2000; Kyne *et al.*, 2001).

The host immune status is also important in determining the outcome of colonisation with *C. difficile*. Immuno-compromised patients are at increased risk for CDI (Yolken *et al.*, 1982) as are patients with a poor immune response to *C. difficile* toxins (Kyne *et al.*, 2000; Mulligan *et al.*, 1993). The role of protective host immunity in determining progression and severity of CDI, specifically the inability to mount an adequate colonic IgA and or serum IgG response to *C. difficile* toxins will be discussed further.

The incidence of CDI acquired in the community appears to be increasing. Cases have also been reported in which there has been no recorded exposure to antibiotics or recent hospitalisation. Similarly an increasing number of cases of CDI are occurring in lower age groups and children. This may be due to the emergence of a new strain that is more commonly associated with animal and non-hospital sources of infection, but this has yet to be fully substantiated (Goorhuis *et al.*, 2008).

4. Microbial virulence factors

4.1 Toxins A and B

The major virulence factors of toxigenic *Clostridium difficile* are two large protein toxins A (TcdA) and B (TcdB) that have both been shown to be responsible for the profound intestinal inflammatory response seen in CDI (Kuehne *et al.*, 2010; Thelestam & Chaves-Olarte, 2000). Toxins A and B are very similar in structure, with three functional domains: a receptor binding domain, a translocation domain and a catalytic domain involved in binding to the host cell, entry into the cytoplasm and inactivation of Rho GTPases respectively (von Eichel-Streiber *et al.*, 1996).

Most disease-causing *C. difficile* strains produce both toxins, encoded by the pathogenicity locus (PaLoc) (Braun *et al.*, 1996; Rupnik *et al.*, 2005). Genetic variations in the toxin genes leads to strains of different 'toxinotypes'(Rupnik, 2008). Most variation across toxinotypes is seen in the binding domain of toxin A, which can lead to so called TcdA-TcdB+ strains which have been found to demonstrate comparable cytotoxicity to normal toxin-producing strains and have been involved in clinical outbreaks (Drudy *et al.*, 2007). Up to 31 different toxinotypes have been identified so far (Rupnik, Feb 2011), highlighting the possibility of a

wide variety of *C. difficile* toxin protein structures and cytotoxic activities. Infection with non-toxigenic strains that do not possess the pathogenicity locus can occur and is usually thought to result in asymptomatic colonisation.

The PaLoc also contains genes involved in regulation of transcription of the toxins (Braun *et al.*, 1996; Rupnik *et al.*, 2005). The epidemic strain BI/NAP-1/ribotype 027 has been shown to contain an 18bp deletion in one of these regulatory genes, *tcdC,* which results in increased toxin production (Dupuy *et al.*, 2008). This is thought to contribute to the virulence of this strain and its involvement in recent epidemics (Warny *et al.*, 2005).

Toxins A and B mediate their cytopathic effect by disrupting the cytoskeleton of intestinal epithelial cells and causing tight junctions to open, resulting in loss of integrity of the protective monolayer (Figure 2).

Once the intestinal epithelium is breached, the toxins are able to access the underlying lamina propria and come into contact with resident macrophages and circulating peripheral blood mononuclear cells (Pothoulakis, 2000; Thelestam & Chaves-Olarte, 2000).

C. difficile toxins are also capable of inducing the release of several classes of cytokines and neuroimmune pro-inflammatory mediators such as interleukin-8 (Il-8) and tumour necrosis factor-α (TNF- α) from intestinal epithelial cells, macrophages and mast cells. These recruit circulating inflammatory cells such as neutrophils into the site of infection and perpetuate the inflammation and fluid secretion associated with CDI (Pothoulakis, 2000).
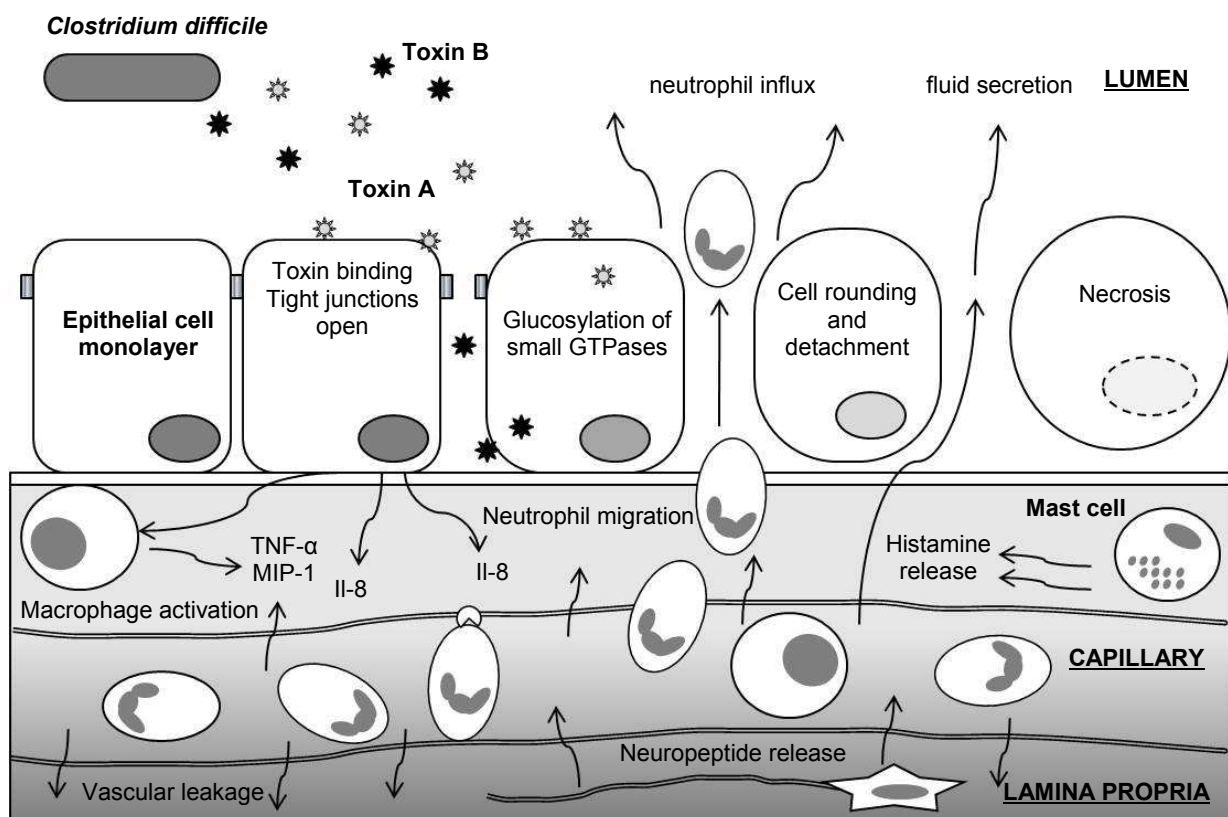

Fig. 2. The role of *C. difficile* toxins A and B in mediating the host inflammatory response

In addition to the indirect effect of the toxins on cells of the immune system, *C. difficile* toxin A has also been shown to directly bind to peripheral blood mononuclear cells, most notably monocytes and induce cell death by apoptosis (Modi *et al.*, 2011; Solomon *et al.*, 2005). This highlights the essential role that toxins play in initiating and prolonging bacterial infection by inactivating key elements of the host protective immune response.

4.2 Binary toxin

In addition to toxins A and B, a further toxigenic component, binary toxin, is expressed in a subset of toxigenic *C. difficile* strains, most notably the epidemic BI/NAP-1/ribotype 027 strain (Rupnik, 2008; Stubbs *et al.*, 2000). Although possessing an ADP-ribosylation function and cytotoxic activity against mammalian cell lines, the role of binary toxin in disease is unclear (Geric *et al.*, 2006).

4.3 Surface layer proteins

In addition to the major secreted toxins, the vegetative *C. difficile* bacterium produces other potential virulence factors. Surface layer proteins are considered to play a vital role in colonisation of the gut and bacterial adherence to the mucosa. They are also important immunogens and can induce the host inflammatory and antibody response (Ausiello *et al.*, 2006; Calabi *et al.*, 2002; Drudy *et al.*, 2004; Pechine *et al.*, 2005). As seen with the toxin proteins, surface layer proteins can be highly variable, particularly surface layer protein A (SlpA) resulting in varying degrees of bacterial adherence ability.

4.4 Flagella

Flagella are also major bacterial virulence factors that enable chemotaxis and penetration of the mucus layer and direct adherence of the bacterium to the epithelial cell surface for localised secretion of toxins. Pathogenic *C. difficile* strains display a range of flagella proteins and subsequent adhesion capabilities (Tasteyre *et al.*, 2001). The immunogenic potential of flagellin is widely appreciated and specific Toll-like receptors (TLRs) are present on the epithelial cell surface to detect flagellin as part of the host cell sensing of pathogens.

5. Host factors that influence outcome of infection

The host's ability to respond to *C. difficile* virulence factors plays a major role in determining the outcome of colonisation with this organism. Infection with the same toxigenic strain can result in a variety of outcomes, including asymptomatic carriage through to symptomatic or severe disease. Certain intrinsic host features, including toxin receptor density (Eglow *et al.*, 1992) and presence or absence of barrier flora (Borriello, 1990) are important in the initial stages of colonisation, however early host detection of infection and initiation of an appropriate immune response likely plays a role in preventing prolonged and severe disease and in providing protection from recurrence of infection.

5.1 Host protective microflora

The most important risk factor for the development of CDI is antimicrobial use, which leads to alteration of the commensal gut microflora and loss of colonisation resistance, enabling

unrestricted growth of toxigenic *C. difficile.* It is proposed that the healthy gut microflora affords resistance by physical inhibition of pathogen adhesion to the mucosa, blocking production of microbial toxins, competition for nutrients and stimulation and development of the mucosal immune system (Mazmanian *et al.*, 2005; Weinstein & Cebra, 1991).

Recent metagenomic studies have provided a detailed insight into the various bacterial species groups involved in host protection against *C. difficile* and have identified an important role for members of the *Bifidobacterium* and *Bacteroides* species (Hopkins & Macfarlane, 2002; Hopkins *et al.*, 2002) Bacterial species diversity was markedly lower in CDI patients and consisted of a higher number of facultative anaerobes than in healthy controls (Hopkins & Macfarlane, 2002).

Healthy resident faecal microflora have been shown to directly suppress the growth of *C. difficile* introduced *in vivo*, possibly through production of volatile fatty acids and bacterial metabolic by-products (Rolfe, 1984). This may explain how approximately 5% of asymptomatic healthy adults carry low concentrations of *C. difficile* in their colon. They have been shown *in vitro* to be held in check by normal gut flora (Fekety & Shah, 1993). Non-toxigenic strains of *C. difficile* may colonise the gut and prevent infection with toxigenic strains (Borriello & Barclay, 1985; Sambol *et al.*, 2002) and even asymptomatic colonisation with toxigenic strains has been associated with a decreased risk of CDI (Shim *et al.*, 1998).

The precise mechanisms by which asymptomatic colonisation is able to protect against CDI have yet to be defined, however continuous immunological challenge with *C. difficile* bacterial peptides and/or low levels of toxins may prime the immune system to act efficiently in response to a subsequent infection (Viscidi *et al.*, 1983). The commensal microflora has also been shown to profoundly influence the development of the humoral components of the gut immune system, influencing IgA production by B cells (Weinstein & Cebra, 1991).

5.2 The innate immune response

0 – 12 hours post-infection

C. difficile toxins act quickly to breach the protective mucosal barrier in order to release nutrients for bacterial growth in to the lumen. The host needs to respond rapidly to circumvent further cellular damage and possible dissemination of toxins into the sub-mucosa and bloodstream. The early host pro-inflammatory response or inducible innate immune response is stimulated initially by the epithelial cells, the first and major cell type encountered by microorganisms in the mucosa.

The intestinal epithelium has developed a wide array of protective mechanisms to prevent bacterial adherence and maintain the integrity of the monolayer, including mucous secretion and tight junctions. Epithelial cells are also able to sense pathogen-associated molecules such as LPS, peptidoglycan and flagellin via specific cell surface TLR receptors. Epithelial cells intoxicated by *C. difficile* release pro-inflammatory mediators including Interleukin-8 (Il-8) and macrophage inflammatory protein-2 (MIP-2) into the underlying lamina propria (Flegel *et al.*, 1991; Mahida *et al.*, 1998).

The sub-mucosal macrophages, monocytes and dendritic cells then disseminate the inflammatory cascade, via the further release of pro-inflammatory cytokines and neuropeptides that recruit peripheral blood cells to the site of infection (Pothoulakis, 1996).

The combined action of cytokines and histamine increases permeability of the vascular endothelium causing fluid leakage and the symptomatic profuse watery diarrhoea associated with *C. difficile* infection. The expression of specific monocyte and leucocyte-adhesion molecules, including CD18 integrins and selectins are upregulated and enable migration of leukocytes and monocytes towards the site of cytokine release (Kelly *et al.*, 1994a). Phagocytosis and digestion of the bacteria with lysozyme, collagenase and peroxidise promotes clearance of the infection, but contributes to the extensive cellular necrosis characterised by the pseudomembraneous plaques.

5.3 The adaptive immune response

1 – 12 days post infection

The importance of the adaptive immune response in influencing the outcome of *C. difficile* colonisation has been appreciated for many years (Aronsson *et al.*, 1985; Johnson *et al.*, 1992; Warny *et al.*, 1994), however the contributions that individual immunoglobulin classes play in protection and prevention have taken longer to elucidate.

An initial challenge with *C. difficile* (irrespective whether this is associated with symptoms or not) will stimulate the maturation and multiplication of naive B-cells, generating antibody-producing plasma cells and specific memory B-cells. The process of immunoglobulin class switching is then induced by specific cytokines, to ensure that the full range of immunoglobulin classes are generated in response to specific *C. difficile* antigens. It is likely that the initial immune challenge occurs in infancy, as approximately 60% of healthy non-colonised or asymptomatic adults have detectable serum IgG and IgA antibodies to *C. diffiicle* toxins (Kelly *et al.*, 1992; Sanchez-Hurtado *et al.*, 2008; Viscidi *et al.*, 1983).

Certain immunoglobulin classes are more important than others in mediating immunity to *C. difficile*. IgA is mainly associated with the gut mucosa and prevention of pathogen colonisation, whereas IgM is released first in infection, followed by IgG, which provides the major protection against pathogens (Underdown & Schiff, 1986). A selective reduction in mucosal IgA has been shown to be associated with severe CDI and reduction in colonic IgA producing cells may predispose to recurrence of infection (Johal *et al.*, 2004).

Serum anti-toxin antibody levels have been found to play an important role in determining the outcome of colonisation and protection against CDI recurrence. Patients who are asymptomatically colonised with *C. difficile* have higher serum anti-toxin A IgG levels than colonised patients who develop diarrhoea (Kyne *et al.*, 2000). This suggests that high anti-toxin A IgG levels at the time of colonistion protect against CDI.

Once colonisation has progressed to CDI, serum IgM and IgG antibody responses to *C. difficile* toxins A, B and non-toxin antigens have been shown to be higher in patients who experience a single episode of CDI compared to patients with recurrent CDI (Kyne *et al.*, 2001). The importance of varying antibody levels during the course of infection was also highlighted, as early IgM responses and later IgG responses (day 3 and day 12 post onset of diarrhoea respectively) were significantly higher in those patients who did not experience recurrence of CDI (Kyne *et al.*, 2001). Deficiency in certain subclasses of IgG (IgG2 and IgG3) has also been found to be related to recurrence of disease (Katchar *et al.*, 2007). A high

natural anti-toxin antibody response does not always however protect from symptomatic CDI. Patients who are critically ill are less likely to be protected than those who are less severely ill, despite similar antibody levels (Kyne *et al.*, 2000). This suggests that other host factors are also important in immune protection and should be considered when predicting outcome of colonisation on host immune status alone.

Immunity to *C. difficile* surface layer proteins (SLP's) would be expected to provide important protection during bacterial colonisation and prevention of symptomatic CDI. IgM, IgA and IgG antibody levels to SLP's were shown to be similar in control patients, asymptomatic carriers and CDI patients, however a lower anti-SLP IgM response was observed in patients with recurrent CDI compared to patients with a single episode of CDI (Drudy *et al.*, 2004). A separate study showed that anti-SLP IgG levels in patients with CDI and asymptomatic carriers were higher compared to levels in control patients without CDI (Sanchez-Hurtado *et al.*, 2008).

C. difficile bacterial flagellar proteins FliC and FliD, and the other surface-associated proteins expressed during the course of infection, have also been shown to be immunogenic. Antibodies against these proteins may be detected after CDI diagnosis and for at least 2 weeks following diagnosis (Pechine *et al.*, 2005).

5.4 Genetic predisposition to infection: Il-8 promoter mutations

Mucosal Il-8 and neutrophil recruitment have been shown to be essential for the pathogenesis of CDI and subsequent amplification of the acute inflammatory response. In particular, the neutrophil response to *C difficile* toxins is likely to play a role in determining the severity of CDI.

C. difficile toxins induce monocytes and epithelial cells to produce Il-8, by increasing binding of nuclear factors to the Il-8 promoter and up-regulating transcription of the Il-8 gene (Linevsky *et al.*, 1997). Some patients may have a genetic pre-disposition to severe CDI due to a single nucleotide polymorphism (SNP) in the Il-8 gene promoter. The presence of an AA (rather than AT or TT) genotype at position −251 has been shown to be associated with increased susceptibility to *C. difficile* toxins and increased fecal Il-8 levels (Jiang *et al.*, 2006; Jiang *et al.*, 2007). The Il-8 promoter AA genotype SNP is also associated with the lack of a protective adaptive antitoxin A antibody response in hospital patients with CDI (Jiang *et al.*, 2006; Jiang *et al.*, 2007).

6. Treatment strategies for CDI

6.1 Antimicrobial therapy

Non-severe CDI can be treated initially with metronidazole for 10-14 days (ASHP, 1998). For severe disease, vancomycin treatment for 10-14 days is suggested. Reliance on antimicrobial agents for treatment of *C. difficile* infection would be expected to further damage the host microflora, increasing the risk of recurrence. Recurrence can occur in up to 25% of cases (Kelly *et al.*, 1994b) and may be due to persistence of initial infection after treatment or re-infection. Treatment for recurrences may follow vancomycin administration in a pulsed regimen (Mc Farland *et al.*, 2002) that allows the germination of spores in between antimicrobial administration to improve efficacy. Other antibiotics, nitazoxanide,

teicoplanin and most recently fidaxomicin have been shown to be as effective as vancomycin treatment and have been indicated as possible alternative therapies (de Lalla *et al.*, 1992 ; Louie *et al.*; Musher *et al.*, 2009).

Non-antimicrobial approaches to management of CDI and in preventing colonisation, such as immune-mediated therapy would limit the effect on the gut microflora, lessening the risk of replapse.

Currently available antimicrobial and non-antimicrobial therapies in use for management of CDI and those under development are outlined in Table 1.

	Currently used	Under development
Antimicrobial agents	Metronidazole Vancomycin Nitazoxanide Teicoplanin Fidaxomicin	Ramplanin
Non-antimicrobial agents		
Toxin neutralising agents	Cholestyramine	Bovine whey protein 'Mucomilk' Tolevamer
Biotherapeutic agents	*Saccharomyces boulardii* Fecal transplants	Non-toxigenic *C. difficile*
Immune-mediated agents	Intravenous immunoglobulin (IVIG)	Human monoclonal antibodies (HuMabs) Toxoid vaccines (Toxins A & B) Active vaccines (SLP, flagella antigens)

Table 1. Treatment strategies for the management of CDI

7. Immune-mediated therapy

An effective antibody response to *C. difficile* toxins and non-toxin antigens is important in influencing the outcome of colonisation and symptomatic CDI and provides the basis for the development of antibody-mediated therapeutics and vaccines. An overview of immunisation strategies in CDI treatment and prevention has been examined in various studies and is reviewed below and illustrated in Figure 3.

7.1 Passive immunisation therapy

Passive immunotherapy, involving the direct transfer of antibodies has been studied in both humans and animals and has mainly focused on antibodies against *C. difficile* toxins A and B

7.1.1 Animal studies

Oral and parenteral administration of bovine anti-toxoid immunoglobulin concentrate (BIC) has been shown to prevent diarrhoea and death in both the hamster and mouse models of CDI (Kelly *et al.*, 1996; Lyerly *et al.*, 1991). Similarly, hen IgY antibodies to recombinant

peptides of both toxins A an B have also been shown to prevent diarrhoea or death when administered orally to hamsters (Kink & Williams, 1998).

Parenteral immunisation with monoclonal antibodies against the binding region of toxin A was able to protect gnotobiotic mice from diarrhoea and death following toxigenic *C. difficile* challenge (Corthier *et al.*, 1991). The best protection against CDI and recurrence is however afforded by combination therapy with neutralising antibodies against both toxins A and B. Human monoclonal antibodies (HuMabs) have been developed after immunisation of human immunoglobulin gene transgenic mice with inactivated toxins A and B (Babcock *et al.*, 2006). Intraperitoneal administration of anti toxin A and B HuMabs protected hamsters from mortality and recurrence of CDI.
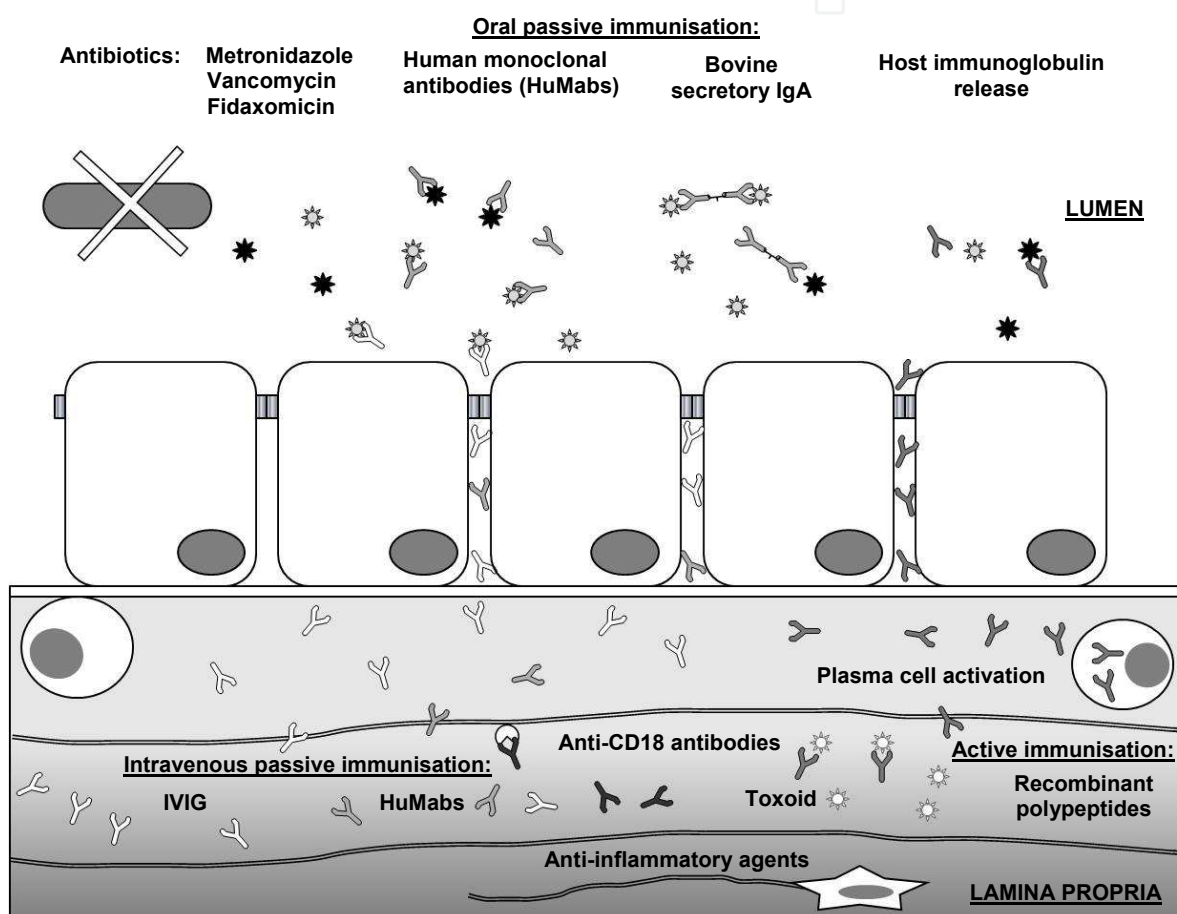

Fig. 3. An overview of immunisation, both active and passive and vaccination against *C. difficile.*

7.1.2 Human studies

Initial studies examining the efficacy of oral administration of bovine immunoglobulin (BIC) in preventing or treating human CDI were promising, but have not progressed further (Kelly *et al.*, 1996; Warny *et al.*, 1999). Bovine anti-*C. difficile* IgA purified from the milk of immunised cows (Mucomilk) was also found to be highly effective at reducing recurrent human CDI, but studies were also not continued further (Mattila *et al.*, 2008; van Dissel *et al.*, 2005).

Intravenous immunisation with pooled anti-toxin A and B IgG immunoglobulin (IVIG) has been the most widely studied mode of passive immunotherapy in humans (Abougergi *et al.*, 2010; O'Horo & Safdar, 2009; Wilcox, 2004). Administration of IVIG to children with low antitoxin antibody levels and relapsing CDI resulted in an increase in antibody levels and subsequent resolution of CDI (Leung *et al.*, 1991). IVIG has also been used for the treatment of severe or refractory CDI with varying success. There is a lack of consensus regarding the optimal dose (150 – 400mg/kg) and dosing regimen (1-3 doses) of IVIG. The lack of randomised controlled clinical trials for IVIG and an association with increased mortality when administered to critically ill patients with CDI in observational studies has served to limit its attractiveness as a possible therapy (Abougergi *et al.*, 2010).

Passive intravenous immunotherapy with HuMabs against toxins A and B has been shown to be well tolerated and effective in protecting patients from recurrence of CDI (Babcock *et al.*, 2006; Leav *et al.*, 2010; Lowy *et al.*, 2010). When used in symptomatic CDI patients in conjunction with standard antimicrobial therapy, recurrence rates were reduced by 72%, although severity of symptoms was not reduced (Lowy *et al.*, 2010). The circulating HuMabs provided protection from subsequent challenge or recurrence due to a half-life of up to 26 days. A role for HuMabs in reducing symptomatic CDI is less well defined however they may be useful as prophylaxis for patients at high risk of infection or in enabling clearance of persistent infection.

7.2 Active immunisation/vaccination

As soon as the importance of *C. difficile* toxins in initiating symptomatic infection was appreciated, studies into active immunisation with inactivated toxin protein toxoids as a means of protecting from CDI were carried out (Kim *et al.*, 1987; Libby *et al.*, 1982).

7.2.1 Animal studies

Hamsters vaccinated with formalin-inactivated *C. difficile* culture filtrate by combined parenteral and mucosal immunisation were found to be protected from CDI (Torres *et al.*, 1995). Similarly, parenteral administration of a toxoid vaccine to hamsters induced high serum levels of anti-toxin A and B antibodies that mediated immune protection against diarrhoea and death (Giannasca *et al.*, 1999). Anti-toxin antibodies were therefore confirmed as the major mediators of the protective response.

Vaccination of mice via transcutaneous administration of a toxin A toxoid derivative and cholera toxin as an immunoadjuvant also showed good induction of anti-toxin A IgG antibodies (Ghose *et al.*, 2007).

Subsequent studies have focused on the use of recombinant polypeptides from toxin A, thought at the time to be the main toxin mediator of intestinal injury in CDI. Immunisation with recombinant toxin A binding domain polypeptides was shown to partially protect hamsters against CDI, and induced a systemic neutralizing immune response in mice, when administered with an *E. coli* toxin adjuvant (Lyerly *et al.*, 1990; Ward *et al.*, 1999). Oral immunisation with an attenuated *Vibrio cholerae* live vector modified to express a fusion protein of the binding domain of toxin A was also shown to induce effective immunity against toxin A in rabbits (Ryan *et al.*, 1997). A DNA vaccine against the binding domain of

toxin A has also been developed for human use. It was shown in a trial to be successfully expressed in host mice cells and induced the production of neutralising antibodies and protected from death (Gardiner *et al.*, 2009).

7.2.2 Human studies

Only one candidate *C. difficile* vaccine has progressed to human trials. A formalin-inactivated toxin A and B toxoid vaccine was safely tolerated in healthy individuals after intramuscular administration and was shown to induce high serum anti-toxin antibody levels (Aboudola *et al.*, 2003; Kotloff *et al.*, 2001). When used to treat patients with recurrent CDI, the vaccine was also successful (Sougioultzis *et al.*, 2005). Phase II clinical trials of a *C. difficile* toxoid vaccine are currently in progress. It is thought that this vaccine would be most cost effective when targeted at those at high-risk of developing CDI and in preventing recurrence thereby reducing the economic burden of *C. difficile* disease (Lee *et al.*, 2010).

7.3 Innate immunity-mediated therapy

The inflammatory response to CDI is mediated in the early stages by the innate immune response, most notably the release of Il-8 from intoxicated epithelial cells and the infiltration of neutrophils from the bloodstream. Pre-treatment of rabbits with monoclonal anti-CD18 antibodies was found to protect from severe inflammation and tissue necrosis, by preventing neutrophils from migrating across the vascular endothelium (Kelly *et al.*, 1994a). *C. difficile* toxins would still be able to cause intestinal epithelial damage in anti-CD18-treated patients via triggering infiltration of other inflammatory cells including monocytes and mast cells via Il-8 release from intoxicated intestinal epithelial cells. Amplification of the inflammatory response by neutrophils however would be greatly reduced. The symptoms of CDI in anti-CD18-treated patients would therefore be expected to be less severe and disease may resolve independently of treatment.

Inflammatory mediators have also been shown to reduce intestinal injury in CDI by blocking signals that perpetuate the inflammatory cascade (Kim *et al.*, 2007; Kokkotou *et al.*, 2009). Their use in conjunction with antibiotics or other therapies may be more effective in the most severe CDI cases.

8. Future strategies for prevention and treatment of CDI

Vaccination strategies currently under trial focus on inducing an immune response to *C. difficile* toxins A and B, however colonisation is the first step in pathogenesis. Other *C. difficile* targets, such as surface layer proteins and flagella antigens that have been developed as vaccine antigens to inhibit colonisation have proven to reduce *C. difficile* colonisation in mice and hamsters, but did not prevent it completely (Brun *et al.*, 2008; Ni Eidhin *et al.*, 2008; Pechine *et al.*, 2007). Widespread vaccination against *C. difficile* would be expected to reduce bacterial carriage and therefore spread of the organism, but may serve to inadvertently reduce other protective members of the *Clostridium* spp. in the gut microflora leading to unanticipated side-effects.

Development of toxoid vaccines against both toxin A and B should also take into account the variety in toxin protein structures exhibited by the 31 toxinotypes currently identified.

All vaccines currently under trial are based on inactivated toxoids from a reference strain of *C. difficile* (Aboudola *et al.*, 2003; Sougioultzis *et al.*, 2005), therefore would in theory only protect immunised patients from a proportion of the possible toxin proteins that would be encountered in infection with multiple strain types. This limited cross-reactivity could be further reduced in vaccines where recombinant polypeptides from the cell-binding domain have been used. This would be most important in recurrent cases, where a small proportion of patients can be infected with a different strain type to their initial infection, rather than re-infection or lack of clearance of their existing colonising strain.

The impact of aging on the efficient functioning of the immune system is also under-investigated with respect to CDI. Older patients are most at risk of developing CDI and therefore are the population most likely to be targeted for vaccination. Due to immune senescence many older patients may not mount an adequate antibody response to vaccination (Burns, 2004).

9. Conclusions

The outcome of *C. difficile* infection, whether it is asymptomatic colonisation or fulminant colitis, is mediated at every stage by the host immune system. The innate immune response plays the foremost role in CDI-induced inflammation and tissue injury, whilst the adaptive immune response mainly mediates protection, either from initial infection or subsequent recurrence. The most effective therapies against *C. difficile* would therefore augment a specific immunoglobulin response whilst suppressing the innate response that triggers neutrophil migration into the mucosa. Vaccine targets developed to protect the at-risk population have shown initial efficacy, however further trials are needed to determine whether they have the widespread cross-reactivity needed to protect patients from all possible strains of *C. difficile*. It also remains to be seen whether the majority of older patients will be able to mount an immune response of a sufficient magnitude to the vaccine, to provide adequate protection.

Until these conditions are met, antimicrobials (metronidazole, vancomycin and the newly FDA-approved Fidaxomicin) remain the mainstay of CDI treatment despite their limitations.

10. References

Aboudola, S., Kotloff, K. L., Kyne, L., Warny, M., Kelly, E. C., Sougioultzis, S., Giannasca, P. J., Monath, T. P. & Kelly, C. P. (2003). *Clostridium difficile* vaccine and serum immunoglobulin G antibody response to toxin A. *Infect Immun* 71, 1608-1610.

Abougergi, M. S., Broor, A., Cui, W. & Jaar, B. G. (2010). Intravenous immunoglobulin for the treatment of severe *Clostridium difficile* colitis: an observational study and review of the literature. *J Hosp Med* 5, E1-9.

Aronsson, B., Granstrom, M., Mollby, R. & Nord, C. E. (1985). Serum antibody response to *Clostridium difficile* toxins in patients with *Clostridium difficile* diarrhoea. *Infection* 13, 97-101.

ASHP (1998). ASHP therapeutic position statement on the preferential use of metronidazole for the treatment of *Clostridium difficile*-associated disease. *Am J Health Syst Pharm* 55, 1407-1411.

Ausiello, C. M., Cerquetti, M., Fedele, G., Spensieri, F., Palazzo, R., Nasso, M., Frezza, S. & Mastrantonio, P. (2006). Surface layer proteins from *Clostridium difficile* induce inflammatory and regulatory cytokines in human monocytes and dendritic cells. *Microbes Infect* 8, 2640-2646.

Babcock, G. J., Broering, T. J., Hernandez, H. J., Mandell, R. B., Donahue, K., Boatright, N., Stack, A. M., Lowy, I., Graziano, R., Molrine, D., Ambrosino, D. M. & Thomas, W. D., Jr. (2006). Human monoclonal antibodies directed against toxins A and B prevent *Clostridium difficile*-induced mortality in hamsters. *Infect Immun* 74, 6339-6347.

Bartlett, J. G. (1994). *Clostridium difficile*: history of its role as an enteric pathogen and the current state of knowledge about the organism. *Clin Infect Dis* 18 Suppl 4, S265-272.

Bignardi, G. E. (1998). Risk factors for *Clostridium difficile* infection. *J Hosp Infect* 40, 1-15.

Borriello, S. P. (1990). The influence of the normal flora on *Clostridium difficile* colonisation of the gut. *Ann Med* 22, 61-67.

Borriello, S. P. & Barclay, F. E. (1985). Protection of hamsters against *Clostridium difficile* ileocaecitis by prior colonisation with non-pathogenic strains. *J Med Microbiol* 19, 339-350.

Braun, V., Hundsberger, T., Leukel, P., Sauerborn, M. & von Eichel-Streiber, C. (1996). Definition of the single integration site of the pathogenicity locus in *Clostridium difficile*. *Gene* 181, 29-38.

Brun, P., Scarpa, M., Grillo, A., Palu, G., Mengoli, C., Zecconi, A., Spigaglia, P., Mastrantonio, P. & Castagliuolo, I. (2008). *Clostridium difficile* TxAC314 and SLP-36kDa enhance the immune response toward a co-administered antigen. *J Med Microbiol* 57, 725-731.

Burns, E. A. (2004). Effects of aging on immune function. *J Nutr Health Aging* 8, 9-18.

Calabi, E., Calabi, F., Phillips, A. D. & Fairweather, N. F. (2002). Binding of *Clostridium difficile* surface layer proteins to gastrointestinal tissues. *Infect Immun* 70, 5770-5778.

Cloud, J., Noddin, L., Pressman, A., Hu, M. & Kelly, C. (2009). *Clostridium difficile* strain NAP-1 is not associated with severe disease in a nonepidemic setting. *Clin Gastroenterol Hepatol* 7, 868-873 e862.

Corthier, G., Muller, M. C., Wilkins, T. D., Lyerly, D. & L'Haridon, R. (1991). Protection against experimental pseudomembranous colitis in gnotobiotic mice by use of monoclonal antibodies against *Clostridium difficile* toxin A. *Infect Immun* 59, 1192-1195.

de Lalla, F., Nicolin, R., Rinaldi, E., Scarpellini, P., Rigoli, R., Manfrin, V. & Tramarin, A. (1992). Prospective study of oral teicoplanin versus oral vancomycin for therapy of pseudomembranous colitis and *Clostridium difficile*-associated diarrhea. *Antimicrob Agents Chemother* 36, 2192-2196.

Dial, S., Kezouh, A., Dascal, A., Barkun, A. & Suissa, S. (2008). Patterns of antibiotic use and risk of hospital admission because of *Clostridium difficile* infection. *CMAJ* 179, 767-772.

Drudy, D., Calabi, E., Kyne, L., Sougioultzis, S., Kelly, E., Fairweather, N. & Kelly, C. P. (2004). Human antibody response to surface layer proteins in *Clostridium difficile* infection. *FEMS Immunol Med Microbiol* 41, 237-242.

Drudy, D., Fanning, S. & Kyne, L. (2007). Toxin A-negative, toxin B-positive *Clostridium difficile*. *Int J Infect Dis* 11, 5-10.

Dupuy, B., Govind, R., Antunes, A. & Matamouros, S. (2008). *Clostridium difficile* toxin synthesis is negatively regulated by TcdC. *J Med Microbiol* 57, 685-689.

Eglow, R., Pothoulakis, C., Itzkowitz, S. & al, e. (1992). Diminished *Clostridium difficile* toxin A sensitivity in newborn rabbit ileum is associated with decreased toxin A receptor. *J Clin Invest* 90, 822-829.

Fekety, R. & Shah, A. B. (1993). Diagnosis and treatment of *Clostridium difficile* colitis. *JAMA* 269, 71-75.

Flegel, W. A., Muller, F., Daubener, W., Fischer, H. G., Hadding, U. & Northoff, H. (1991). Cytokine response by human monocytes to *Clostridium difficile* toxin A and toxin B. *Infect Immun* 59, 3659-3666.

Gardiner, D. F., Rosenberg, T., Zaharatos, J., Franco, D. & Ho, D. D. (2009). A DNA vaccine targeting the receptor-binding domain of *Clostridium difficile* toxin A. *Vaccine* 27, 3598-3604.

Geric, B., Carman, R. J., Rupnik, M., Genheimer, C. W., Sambol, S. P., Lyerly, D. M., Gerding, D. N. & Johnson, S. (2006). Binary toxin-producing, large clostridial toxin-negative *Clostridium difficile* strains are enterotoxic but do not cause disease in hamsters. *J Infect Dis* 193, 1143-1150.

Ghose, C., Kalsy, A., Sheikh, A., Rollenhagen, J., John, M., Young, J., Rollins, S. M., Qadri, F., Calderwood, S. B., Kelly, C. P. & Ryan, E. T. (2007). Transcutaneous immunization with *Clostridium difficile* toxoid A induces systemic and mucosal immune responses and toxin A-neutralizing antibodies in mice. *Infect Immun* 75, 2826-2832.

Giannasca, P. J., Zhang, Z. X., Lei, W. D., Boden, J. A., Giel, M. A., Monath, T. P. & Thomas, W. D., Jr. (1999). Serum antitoxin antibodies mediate systemic and mucosal protection from *Clostridium difficile* disease in hamsters. *Infect Immun* 67, 527-538.

Goorhuis, A., Debast, S. B., van Leengoed, L. A., Harmanus, C., Notermans, D. W., Bergwerff, A. A. & Kuijper, E. J. (2008). *Clostridium difficile* PCR ribotype 078: an emerging strain in humans and in pigs? *J Clin Microbiol* 46, 1157; author reply 1158.

Hopkins, M. J. & Macfarlane, G. T. (2002). Changes in predominant bacterial populations in human faeces with age and with *Clostridium difficile* infection. *J Med Microbiol* 51, 448-454.

Hopkins, M. J., Sharp, R. & Macfarlane, G. T. (2002). Variation in human intestinal microbiota with age. *Dig Liver Dis* 34 Suppl 2, S12-18.

Jiang, Z. D., DuPont, H. L., Garey, K., Price, M., Graham, G., Okhuysen, P., Dao-Tran, T. & LaRocco, M. (2006). A common polymorphism in the interleukin 8 gene promoter is associated with Clostridium difficile diarrhea. *Am J Gastroenterol* 101, 1112-1116.

Jiang, Z. D., Garey, K. W., Price, M., Graham, G., Okhuysen, P., Dao-Tran, T., LaRocco, M. & DuPont, H. L. (2007). Association of interleukin-8 polymorphism and immunoglobulin G anti-toxin A in patients with *Clostridium difficile*-associated diarrhea. *Clin Gastroenterol Hepatol* 5, 964-968.

Johal, S. S., Lambert, C. P., Hammond, J., James, P. D., Borriello, S. P. & Mahida, Y. R. (2004). Colonic IgA producing cells and macrophages are reduced in recurrent and non-recurrent *Clostridium difficile* associated diarrhoea. *J Clin Pathol* 57, 973-979.

Johnson, S. & Gerding, D. N. (1998). *Clostridium difficile*--associated diarrhea. *Clin Infect Dis* 26, 1027-1034; quiz 1035-1026.

Johnson, S., Gerding, D. N. & Janoff, E. N. (1992). Systemic and mucosal antibody responses to toxin A in patients infected with *Clostridium difficile*. *J Infect Dis* 166, 1287-1294.

Katchar, K., Taylor, C. P., Tummala, S., Chen, X., Sheikh, J. & Kelly, C. P. (2007). Association between IgG2 and IgG3 subclass responses to toxin A and recurrent *Clostridium difficile*-associated disease. *Clin Gastroenterol Hepatol* 5, 707-713.

Kelly, C. P., Becker, S., Linevsky, J. K., Joshi, M. A., O'Keane, J. C., Dickey, B. F., LaMont, J. T. & Pothoulakis, C. (1994a). Neutrophil recruitment in *Clostridium difficile* toxin A enteritis in the rabbit. *J Clin Invest* 93, 1257-1265.

Kelly, C. P., Pothoulakis, C. & LaMont, J. T. (1994b). *Clostridium difficile* colitis. *N Engl Jour Med* 330, 257-262.

Kelly, C. P., Pothoulakis, C., Orellana, J. & LaMont, J. T. (1992). Human colonic aspirates containing immunoglobulin A antibody to *Clostridium difficile* toxin A inhibit toxin A-receptor binding. *Gastroenterology* 102, 35-40.

Kelly, C. P., Pothoulakis, C., Vavva, F., Castagliuolo, I., Bostwick, E. F., O'Keane, J. C., Keates, S. & LaMont, J. T. (1996). Anti-*Clostridium difficile* bovine immunoglobulin concentrate inhibits cytotoxicity and enterotoxicity of C. difficile toxins. *Antimicrob Agents Chemother* 40, 373-379.

Kim, H., Rhee, S. H., Pothoulakis, C. & Lamont, J. T. (2007). Inflammation and apoptosis in *Clostridium difficile* enteritis is mediated by PGE2 up-regulation of Fas ligand. *Gastroenterology* 133, 875-886.

Kim, P. H., Iaconis, J. P. & Rolfe, R. D. (1987). Immunization of adult hamsters against *Clostridium difficile*-associated ileocecitis and transfer of protection to infant hamsters. *Infect Immun* 55, 2984-2992.

Kink, J. A. & Williams, J. A. (1998). Antibodies to recombinant *Clostridium difficile* toxins A and B are an effective treatment and prevent relapse of *C. difficile*-associated disease in a hamster model of infection. *Infect Immun* 66, 2018-2025.

Kokkotou, E., Espinoza, D. O., Torres, D., Karagiannides, I., Kosteletos, S., Savidge, T., O'Brien, M. & Pothoulakis, C. (2009). Melanin-concentrating hormone (MCH) modulates *C difficile* toxin A-mediated enteritis in mice. *Gut* 58, 34-40.

Kotloff, K. L., Wasserman, S. S., Losonsky, G. A., Thomas, W., Jr., Nichols, R., Edelman, R., Bridwell, M. & Monath, T. P. (2001). Safety and immunogenicity of increasing doses of a *Clostridium difficile* toxoid vaccine administered to healthy adults. *Infect Immun* 69, 988-995.

Kuehne, S. A., Cartman, S. T., Heap, J. T., Kelly, M. L., Cockayne, A. & Minton, N. P. (2010). The role of toxin A and toxin B in *Clostridium difficile* infection. *Nature* 467, 711-713.

Kyne, L., Warny, M., Qamar, A. & Kelly, C. P. (2000). Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. *N Engl J Med* 342, 390-397.

Kyne, L., Warny, M., Qamar, A. & Kelly, C. P. (2001). Association between antibody response to toxin A and protection against recurrent *Clostridium difficile* diarrhoea. *Lancet* 357, 189-193.

Larson, H. E., Price, A. B., Honour, P. & Borriello, S. P. (1978). *Clostridium difficile* and the aetiology of pseudomembranous colitis. *Lancet* 1, 1063-1066.

Leav, B. A., Blair, B., Leney, M., Knauber, M., Reilly, C., Lowy, I., Gerding, D. N., Kelly, C. P., Katchar, K., Baxter, R., Ambrosino, D. & Molrine, D. (2010). Serum anti-toxin B antibody correlates with protection from recurrent *Clostridium difficile* infection (CDI). *Vaccine* 28, 965-969.

Lee, B. Y., Popovich, M. J., Tian, Y., Bailey, R. R., Ufberg, P. J., Wiringa, A. E. & Muder, R. R. (2010). The potential value of *Clostridium difficile* vaccine: an economic computer simulation model. *Vaccine* 28, 5245-5253.

Leung, D. Y., Kelly, C. P., Boguniewicz, M., Pothoulakis, C., LaMont, J. T. & Flores, A. (1991). Treatment with intravenously administered gamma globulin of chronic relapsing colitis induced by *Clostridium difficile* toxin. *J Pediatr* 118, 633-637.

Libby, J. M., Jortner, B. S. & Wilkins, T. D. (1982). Effects of the two toxins of *Clostridium difficile* in antibiotic-associated cecitis in hamsters. *Infect Immun* 36, 822-829.

Linevsky, J. K., Pothoulakis, C., Keates, S., Warny, M., Keates, A. C., Lamont, J. T. & Kelly, C. P. (1997). IL-8 release and neutrophil activation by *Clostridium difficile* toxin-exposed human monocytes. *Am J Physiol* 273, G1333-1340.

Loo, V. G., Poirier, L., Miller, M. A., Oughton, M., Libman, M. D., Michaud, S., Bourgault, A. M., Nguyen, T., Frenette, C., Kelly, M., Vibien, A., Brassard, P., Fenn, S., Dewar, K., Hudson, T. J., Horn, R., Rene, P., Monczak, Y. & Dascal, A. (2005). A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N Engl J Med* 353, 2442-2449.

Louie, T. J., Miller, M. A., Mullane, K. M., Weiss, K., Lentnek, A., Golan, Y., Gorbach, S., Sears, P. & Shue, Y.-K. Fidaxomicin versus Vancomycin for *Clostridium difficile* Infection. *New England Journal of Medicine* 364, 422-431.

Lowy, I., Molrine, D. C., Leav, B. A., Blair, B. M., Baxter, R., Gerding, D. N., Nichol, G., Thomas, W. D., Jr., Leney, M., Sloan, S., Hay, C. A. & Ambrosino, D. M. (2010). Treatment with monoclonal antibodies against *Clostridium difficile* toxins. *N Engl J Med* 362, 197-205.

Lyerly, D. M., Bostwick, E. F., Binion, S. B. & Wilkins, T. D. (1991). Passive immunization of hamsters against disease caused by *Clostridium difficile* by use of bovine immunoglobulin G concentrate. *Infect Immun* 59, 2215-2218.

Lyerly, D. M., Johnson, J. L., Frey, S. M. & Wilkins, T. D. (1990). Vaccination against lethal *Clostridium difficile* enterocolitis with a nontoxic recombinant peptide of toxin A. *Current Microbiology* 21, 29-32.

Mahida, Y. R., Galvin, A., Makh, S., Hyde, S., Sanfilippo, L., Borriello, S. P. & Sewell, H. F. (1998). Effect of *Clostridium difficile* toxin A on human colonic lamina propria cells: Early loss of macrophages followed by T-cell apoptosis. *Infect Immun* 66, 5462-5469.

Mattila, E., Anttila, V. J., Broas, M., Marttila, H., Poukka, P., Kuusisto, K., Pusa, L., Sammalkorpi, K., Dabek, J., Koivurova, O. P., Vahatalo, M., Moilanen, V. & Widenius, T. (2008). A randomized, double-blind study comparing *Clostridium difficile* immune whey and metronidazole for recurrent *Clostridium difficile*-associated diarrhoea: efficacy and safety data of a prematurely interrupted trial. *Scand J Infect Dis* 40, 702-708.

Mazmanian, S. K., Liu, C. H., Tzianabos, A. O. & Kasper, D. L. (2005). An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 122, 107-118.

Mc Farland, L. V., Elmer, G. W. & Surawicz, C. M. (2002). Breaking the cycle: treatment strategies for 163 cases of recurrent *Clostridium difficile* disease. . *Am J Gastroenterol* 97, 1769.

Modi, N., Gulati, N., Solomon, K., Monaghan, T., Robins, A., Sewell, H. F. & Mahida, Y. R. (2011). Differential binding and internalisation of *Clostridium difficile* toxin A by

human peripheral blood monocytes, neutrophils and lymphocytes. *Scand J Immunol.*

Mulligan, M. E., Miller, S. D., McFarland, L. V., Fung, H. C. & Kwok, R. Y. (1993). Elevated levels of serum immunoglobulins in asymptomatic carriers of *Clostridium difficile*. *Clin Infect Dis* 16 Suppl 4, S239-244.

Musher, D. M., Logan, N., Bressler, A. M., Johnson, D. P. & Rossignol, J.-F. o. (2009). Nitazoxanide versus Vancomycin in *Clostridium difficile* Infection: A Randomized, Double-Blind Study. *Clinical Infectious Diseases* 48, e41-e46.

Ni Eidhin, D. B., O'Brien, J. B., McCabe, M. S., Athie-Morales, V. & Kelleher, D. P. (2008). Active immunization of hamsters against *Clostridium difficile* infection using surface-layer protein. *FEMS Immunol Med Microbiol* 52, 207-218.

O'Horo, J. & Safdar, N. (2009). The role of immunoglobulin for the treatment of *Clostridium difficile* infection: a systematic review. *Int J Infect Dis* 13, 663-667.

Owens, R. C., Jr., Donskey, C. J., Gaynes, R. P., Loo, V. G. & Muto, C. A. (2008). Antimicrobial-associated risk factors for *Clostridium difficile* infection. *Clin Infect Dis* 46 Suppl 1, S19-31.

Pechine, S., Janoir, C., Boureau, H., Gleizes, A., Tsapis, N., Hoys, S., Fattal, E. & Collignon, A. (2007). Diminished intestinal colonization by *Clostridium difficile* and immune response in mice after mucosal immunization with surface proteins of *Clostridium difficile*. *Vaccine* 25, 3946-3954.

Pechine, S., Janoir, C. & Collignon, A. (2005). Variability of *Clostridium difficile* surface proteins and specific serum antibody response in patients with *Clostridium difficile*-associated disease. *J Clin Microbiol* 43, 5018-5025.

Pepin, J., Saheb, N., Coulombe, M. A., Alary, M. E., Corriveau, M. P., Authier, S., Leblanc, M., Rivard, G., Bettez, M., Primeau, V., Nguyen, M., Jacob, C. E. & Lanthier, L. (2005). Emergence of fluoroquinolones as the predominant risk factor for *Clostridium difficile*-associated diarrhea: a cohort study during an epidemic in Quebec. *Clin Infect Dis* 41, 1254-1260.

Pepin, J., Vo, T. T., Boutros, M., Marcotte, E., Dial, S., Dube, S., Vasilevsky, C. A., McFadden, N., Patino, C. & Labbe, A. C. (2009). Risk factors for mortality following emergency colectomy for fulminant *Clostridium difficile* infection. *Dis Colon Rectum* 52, 400-405.

Pothoulakis, C. (1996). Pathogenesis of *Clostridium difficile*-associated diarrhoea. *Eur J Gastroenterol Hepatol* 8, 1041-1047.

Pothoulakis, C. (2000). Effects of *Clostridium difficile* toxins on epithelial cell barrier. *Ann N Y Acad Sci* 915, 347-356.

Rolfe, R. D. (1984). Role of volatile fatty acids in colonization resistance to *Clostridium difficile*. *Infect Immun* 45, 185-191.

Rupnik, M. (2008). Heterogeneity of large clostridial toxins: importance of *Clostridium difficile* toxinotypes. *FEMS Microbiol Rev* 32, 541-555.

Rupnik, M. (Feb 2011). *Clostridium difficile* Toxinotypes, Date of access: 28 June 2011, Available from: http://www.mf.uni-mb.si/mikro/tox/. .

Rupnik, M., Dupuy, B., Fairweather, N. F., Gerding, D. N., Johnson, S., Just, I., Lyerly, D. M., Popoff, M. R., Rood, J. I., Sonenshein, A. L., Thelestam, M., Wren, B. W., Wilkins, T. D. & von Eichel-Streiber, C. (2005). Revised nomenclature of *Clostridium difficile* toxins and associated genes. *J Med Microbiol* 54, 113-117.

Ryan, E. T., Butterton, J. R., Smith, R. N., Carroll, P. A., Crean, T. I. & Calderwood, S. B. (1997). Protective immunity against *Clostridium difficile* toxin A induced by oral immunization with a live, attenuated Vibrio cholerae vector strain. *Infect Immun* 65, 2941-2949.

Sambol, S. P., Merrigan, M. M., Tang, J. K., Johnson, S. & Gerding, D. N. (2002). Colonization for the prevention of *Clostridium difficile* disease in hamsters. *J Infect Dis* 186, 1781-1789.

Sanchez-Hurtado, K., Corretge, M., Mutlu, E., McIlhagger, R., Starr, J. M. & Poxton, I. R. (2008). Systemic antibody response to Clostridium difficile in colonized patients with and without symptoms and matched controls. *J Med Microbiol* 57, 717-724.

Savidge, T. C., Pan, W. H., Newman, P., O'Brien, M., Anton, P. M. & Pothoulakis, C. (2003). *Clostridium difficile* toxin B is an inflammatory enterotoxin in human intestine. *Gastroenterology* 125, 413-420.

Shim, J. K., Johnson, S., Samore, M. H., Bliss, D. Z. & Gerding, D. N. (1998). Primary symptomless colonisation by *Clostridium difficile* and decreased risk of subsequent diarrhoea. *Lancet* 351, 633-636.

Solomon, K., Webb, J., Ali, N., Robins, R. A. & Mahida, Y. R. (2005). Monocytes are highly sensitive to *Clostridium difficile* toxin A-induced apoptotic and nonapoptotic cell death. *Infect Immun* 73, 1625-1634.

Sougioultzis, S., Kyne, L., Drudy, D., Keates, S., Maroo, S., Pothoulakis, C., Giannasca, P. J., Lee, C. K., Warny, M., Monath, T. P. & Kelly, C. P. (2005). *Clostridium difficile* toxoid vaccine in recurrent *C. difficile*-associated diarrhea. *Gastroenterology* 128, 764-770.

Stubbs, S., Rupnik, M., Gibert, M., Brazier, J., Duerden, B. & Popoff, M. (2000). Production of actin-specific ADP-ribosyltransferase (binary toxin) by strains of *Clostridium difficile*. *FEMS Microbiol Lett* 186, 307-312.

Sunenshine, R. H. & McDonald, L. C. (2006). *Clostridium difficile*-associated disease: new challenges from an established pathogen. *Cleve Clin J Med* 73, 187-197.

Tasteyre, A., Barc, M. C., Collignon, A., Boureau, H. & Karjalainen, T. (2001). Role of FliC and FliD flagellar proteins of *Clostridium difficile* in adherence and gut colonization. *Infect Immun* 69, 7937-7940.

Thelestam, M. & Chaves-Olarte, E. (2000). Cytotoxic effects of the *Clostridium difficile* toxins. *Curr Top Microbiol Immunol* 250, 85-96.

Torres, J. F., Lyerly, D. M., Hill, J. E. & Monath, T. P. (1995). Evaluation of formalin-inactivated *Clostridium difficile* vaccines administered by parenteral and mucosal routes of immunization in hamsters. *Infect Immun* 63, 4619-4627.

Underdown, B. J. & Schiff, J. M. (1986). Immunoglobulin-A - Strategic defense initiative at the mucosal surface. *Annu Rev Immunol* 4, 389-417.

van Dissel, J. T., de Groot, N., Hensgens, C. M., Numan, S., Kuijper, E. J., Veldkamp, P. & van 't Wout, J. (2005). Bovine antibody-enriched whey to aid in the prevention of a relapse of *Clostridium difficile*-associated diarrhoea: preclinical and preliminary clinical data. *J Med Microbiol* 54, 197-205.

Viscidi, R., Laughon, B. E., Yolken, R., Bo-Linn, P., Moench, T., Ryder, R. W. & Bartlett, J. G. (1983). Serum antibody response to toxins A and B of *Clostridium difficile*. *J Infect Dis* 148, 93-100.

von Eichel-Streiber, C., Boquet, P., Sauerborn, M. & Thelestam, M. (1996). Large clostridial cytotoxins--a family of glycosyltransferases modifying small GTP-binding proteins. *Trends Microbiol* 4, 375-382.

Ward, S. J., Douce, G., Dougan, G. & Wren, B. W. (1999). Local and systemic neutralizing antibody responses induced by intranasal immunization with the nontoxic binding domain of toxin A from *Clostridium difficile*. *Infect Immun* 67, 5124-5132.

Warny, M., Fatimi, A., Bostwick, E. F., Laine, D. C., Lebel, F., LaMont, J. T., Pothoulakis, C. & Kelly, C. P. (1999). Bovine immunoglobulin concentrate-*Clostridium difficile* retains *C. difficile* toxin neutralising activity after passage through the human stomach and small intestine. *Gut* 44, 212-217.

Warny, M., Pepin, J., Fang, A., Killgore, G., Thompson, A., Brazier, J., Frost, E. & McDonald, L. C. (2005). Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *Lancet* 366, 1079-1084.

Warny, M., Vaerman, J. P., Avesani, V. & Delmee, M. (1994). Human antibody response to *Clostridium difficile* toxin A in relation to clinical course of infection. *Infect Immun* 62, 384-389.

Weinstein, P. D. & Cebra, J. J. (1991). The preference for switching to IgA expression by Peyer's patch germinal center B cells is likely due to the intrinsic influence of their microenvironment. *J Immunol* 147, 4126-4135.

Wilcox, M. H. (2004). Descriptive study of intravenous immunoglobulin for the treatment of recurrent *Clostridium difficile* diarrhoea. *J Antimicrob Chemother* 53, 882-884.

Yolken, R. H., Bishop, C. A., Townsend, T. R., Bolyard, E. A., Bartlett, J., Santos, G. W. & Saral, R. (1982). Infectious gastroenteritis in bone-marrow-transplant recipients. *N Engl J Med* 306, 1010-1012.
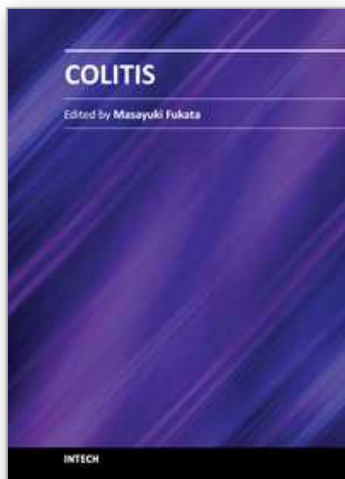
Colitis

Edited by Dr Fukata

ISBN 978-953-307-799-4

Hard cover, 196 pages

Publisher InTech

Published online 05, January, 2012

Published in print edition January, 2012

Inflammation of the colon is collectively called "Colitis". Since a variety of conditions may cause colitis and its manifestations are similar among the causes, selection of the right treatment based on the correct diagnosis is important in the management of this group of illnesses. Over the last few decades, a major shift has been observed in the clinical attention to the pathogenesis of colitis from infectious to idiopathic inflammatory bowel diseases. Colitis cases that are associated with chemical therapeutics and specific pathogens such as amoeba, have become prominent in hospitalized individuals and immune deficient patients, respectively. In addition, a great deal of progress has been made in colitis research triggering the need for updating our knowledge about colitis. This book Colitis provides comprehensive information on the pathogenesis, mechanism of resolution, and treatment strategies of colitis.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Katie Solomon and Lorraine Kyne (2012). Clostridium difficile-Associated Colitis: Role of the Immune Response and Approach to Treatment, Colitis, Dr Fukata (Ed.), ISBN: 978-953-307-799-4, InTech, Available from: http://www.intechopen.com/books/colitis/clostridium-difficile-associated-colitis-role-of-the-immune-response-and-approach-to-treatment

INTECH
open science | open minds