Review

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The host immune response to *Clostridium difficile*

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Clostridium difficile is the most common cause of nosocomial bacterial diarrhoea in the Western world. Diarrhoea and colitis are caused by the actions of toxins A and B released by pathogenic strains of *C. difficile*. Adaptive immune responses to these toxins influence the outcomes of *C. difficile* infection (CDI). Symptomless carriers of toxinogenic *C. difficile* and those with a single episode of CDI without recurrence show more robust antitoxin immune responses than those with symptomatic and recurrent disease. Immune-based approaches to CDI therapy and prevention have been developed using active vaccination or passive immunotherapy targeting *C. difficile* toxins. Innate immune responses to *C. difficile* and its toxins are also central to the pathophysiology of CDI. An acute intestinal inflammatory response with prominent neutrophil infiltration and associated tissue injury is characteristic of CDI. Furthermore, inhibiting this acute inflammatory response can protect against the intestinal injury that results from exposure to *C. difficile* toxins in animal models. Studies examining host risk factors for CDI have led to validated clinical prediction tools for risk of primary and of recurrent disease. Risk factors associated with severe CDI with poor clinical outcomes have also been identified and include marked elevation of the peripheral white blood cell count and elevated creatinine. However, further work is needed in this area to guide the clinical application of new approaches to disease prevention and treatment including new antimicrobials as well as passive and active immunization.

Introduction

Clostridium difficile infection (CDI) usually occurs as a complication of nosocomial antibiotic use and is mediated by toxins A and B released by pathogenic strains of the bacterium. The host response to CDI varies from symptomless carriage, to mild self-limited diarrhoea, to recurrent CDI, to severe, life-threatening, colitis and, in increasing numbers of cases, to death (Kelly & LaMont, 2008). There is now considerable evidence to demonstrate that host immune and inflammatory responses contribute in large part to these different disease outcomes. Age, underlying and co-morbid disease, drug therapy and adaptive and innate immune responses all influence CDI severity and course. This review will examine several aspects of the host response to CDI, with an emphasis on the adaptive immune response, as this has been a focus of recent advances in our understanding of disease pathogenesis and is a very active area for new drug development. The potentially crucial role played by innate immune responses in CDI has drawn relatively little attention from clinical researchers but may prove to be an important target for adjuncts to antibiotics, especially in severe disease. Finally, we will discuss clinical prediction tools for CDI as a practical means to harness our advancing knowledge of host factors that determine disease outcomes.

The ability to accurately identify those at highest risk for severe or recurrent disease will allow us to target current and emerging interventions to prevent and more effectively treat CDI.

Natural adaptive immunity to *C. difficile* and its toxins

Antibodies to *C. difficile* in the general population

Many healthy older children and adults (~60 %) have detectable serum IgG and IgA antibodies to *C. difficile* toxins A and B, even in the absence of *C. difficile* colonization or active infection (Kelly *et al.*, 1992; Nakamura *et al.*, 1981; Sánchez-Hurtado *et al.*, 2008; Viscidi *et al.*, 1983). It is likely that antibody production is stimulated in infancy and perpetuated through adult life by environmental exposure to *C. difficile* itself or to other clostridial species, such as *Clostridium sordellii*, which possesses cross-reacting antigens (Viscidi *et al.*, 1983).

Antibodies to *C. difficile* and asymptomatic carriage

Early studies suggested that immune responses to *C. difficile* and its toxins played a role in disease presentation and course (Aronsson *et al.*, 1985; Johnson *et al.*, 1992;

Mulligan *et al.*, 1993; Warny *et al.*, 1994). We subsequently tested this hypothesis in a prospective study of 271 hospitalized antibiotic recipients in whom we measured serum and faecal antibody responses to *C. difficile* toxins A, B and non-toxin antigens over time (Kyne *et al.*, 2000). We found that at the time of colonization, asymptomatic carriers of *C. difficile* had significantly higher serum IgG antibody levels to toxin A compared with colonized patients who later developed diarrhoea. Importantly, serum IgG antitoxin A levels were also significantly correlated with IgG levels against toxin B and non-toxin antigens, which were also higher in asymptomatic carriers; however, this difference was not statistically significant, possibly due to small sample sizes. Serum antibody levels were not affected by age, co-morbidity or severity of underlying disease; however, the effect of higher serum IgG antitoxin A levels in protecting against diarrhoea was less marked in patients who were severely ill. This moderation of the effects of protective immunity in severely ill patients may have implications for future immunotherapy-based treatments, which may be less effective in very ill patients. Faecal antibodies were not associated with protection against symptoms in this study but were in a previous study (Kyne *et al.*, 2000; Warny *et al.*, 1994).

More recently, Sánchez-Hurtado *et al.* (2008) found that serum IgG and IgM antibody levels against crude and purified toxin A were similar in colonized patients with and without symptoms and matched controls. However, the authors acknowledge that they were unable to establish the time of colonization and that antibody levels may change over time, as has been demonstrated (Kyne *et al.*, 2000).

Antibodies to *C. difficile* and recurrent disease

In patients with CDI, IgM levels against toxins A, B and non-toxin antigens, measured on the third day after the initial onset of diarrhoea, were significantly higher in patients who had a single episode of diarrhoea than in patients who later developed recurrent CDI (Kyne *et al.*, 2001). Higher day 12 IgG antibodies to toxin A were also associated with protection against recurrence. Katchar *et al.* (2007) found that IgG2 and IgG3 subclasses of antitoxin A IgG antibodies were deficient in patients with recurrent CDI.

Taken together, these studies suggest that patients who become colonized with *C. difficile* who can boost an anamnestic systemic immune response to *C. difficile* toxins are less likely to develop symptoms. Likewise, symptomatic patients who can mount an immune response early in the course of their illness are less likely to have recurrent CDI. The immune responses to toxin A, B and non-toxin antigens are correlated. This observation is important as effective protection against initial symptoms or recurrence is likely to involve strategies aimed at inducing immunity to both toxins and to non-toxin antigens.

Antibody responses to non-toxin antigens

To further evaluate the role of the host immune response to *C. difficile* antigens, other than toxins A and B, several investigators have examined the role of antibody response to surface-layer proteins (SLPs) (Drudy *et al.*, 2004; Sánchez-Hurtado *et al.*, 2008; Wright *et al.*, 2008). SLPs have been shown to have *in vitro* and *in vivo* adhesive properties and to play a role in bacterial adhesion (Calabi *et al.*, 2002). Therefore, they may play an important role in intestinal colonization and persistence of infection with *C. difficile*. Drudy *et al.* (2004) found that antibody levels to SLPs were similar in patients with CDI, asymptomatic carriers and controls. However, patients with recurrent CDI failed to mount an IgM immune response to SLPs compared to patients with a single episode of CDI. In another study, IgG but not IgM levels to SLPs were similar in cases and carriers, but higher compared to controls (Sánchez-Hurtado *et al.*, 2008). Outcomes in terms of recurrence were not examined in that study. Péchiné *et al.* (2005a) found that the bacterial flagellar proteins FliC and FliD, and the surface-associated proteins Cwp66 and Cwp84, were expressed during the course of infection and that antibodies against these proteins could be detected after CDI diagnosis and for at least 2 weeks.

Immunization against *C. difficile*

The results of the observational studies presented above demonstrate the relevance of antibodies both against toxin and against non-toxin antigens in influencing the outcomes of CDI. These studies have also provided scientific rationale for the development of antibody products and vaccines against *C. difficile*. The efficacy of active and passive immunity to *C. difficile* toxins in protection against disease has been explored in various small animal models of CDI and also in humans. Vaccines against surface proteins, aimed at preventing or reducing colonization, are also being developed and tested in animals.

Passive immunization against *C. difficile* toxins in animals

Oral and parenteral administration of antitoxin antibody formulations has been shown to protect against diarrhoea and death in hamsters and mice. Anti-*C. difficile* bovine immunoglobulin concentrate (BIC), prepared from the colostrum of cows immunized with culture filtrate toxoid, protected against diarrhoea and death in hamsters before and during clindamycin/*C. difficile* challenge (Kelly *et al.*, 1996; Lyerly *et al.*, 1991). Kink & Williams (1998) immunized hens, with various recombinant peptides spanning the entire *C. difficile* toxin proteins, for the production of egg IgY antibodies, which were administered orogastrically to hamsters. They found that neutralizing antibodies to both toxins A and B provided complete protection from diarrhoea and death in this model.

Parenteral passive immunotherapy is also effective in animals. In one study, Corthier *et al.* (1991) administered a monoclonal antibody against the putative binding region of toxin A to gnotobiotic mice. The mice were protected against diarrhoea and death following challenge with toxinogenic *C. difficile*. More recently, Babcock *et al.* (2006) developed neutralizing, fully human monoclonal antibodies (HumAbs) directed against either toxin A or toxin B by immunizing mice transgenic for human immunoglobulin genes with inactivated toxins. Antitoxin A HumAbs reduced mortality in the hamster model; however combination intraperitoneal administration of HumAbs against both toxin A and B resulted in the best protection against mortality and recurrence.

Active immunization against *C. difficile* toxins in animals

Protection against lethal CDI by active immunization with *C. difficile* toxoids was shown in hamsters as early as the 1980s (Fernie *et al.*, 1983; Kim *et al.*, 1987; Libby *et al.*, 1982). Torres *et al.* (1995) later found that combined parenteral and mucosal immunization with a formalin-inactivated culture-filtrate *C. difficile* vaccine provided complete protection against CDI in hamsters. Using a more purified toxoid preparation, Giannasca *et al.* (1999) demonstrated that parenteral administration induced high levels of serum toxin A and B neutralizing activity and protected hamsters against diarrhoea and death. Immunity could also be transferred to naïve hamsters using serum or ascites, demonstrating that circulating antitoxin antibodies mediated the effector functions in this model of CDI.

While these previous animal models examined parenteral and mucosal immunization routes, a more recent study assessed whether transcutaneous immunization (TCI) could induce immune responses against toxin A in mice (Ghose *et al.*, 2007). In a novel approach to immunization against *C. difficile*, the investigators demonstrated that TCI with a toxoid derivative of toxin A and immunoadjuvant cholera toxin induced serum anti-*C. difficile* toxin A IgG responses.

Recombinant antigens have also been examined as potential vaccine candidates. Immunization with recombinant polypeptides from the cell-binding domain of toxin A partially protected against diarrhoea and death in hamsters (Lyerly *et al.*, 1990). In a murine model, these recombinant polypeptides induced local and systemic neutralizing antibody responses after intra-nasal immunization along with adjuvant heat-labile toxin from *Escherichia coli* (Ward *et al.*, 1999). Various attenuated live vectors have been investigated in an attempt to improve delivery of these recombinant polypeptides to the intestinal mucosa. Ryan *et al.* (1997) found that protective immunity against *C. difficile* toxin A (TcdA) was induced in rabbits by oral administration of the nontoxic repeated binding sequence of toxin A expressed as a fusion protein in an attenuated *Vibrio cholerae* vector strain.

A recent development in active immunization of animals has been the development of a DNA vaccine targeting the receptor-binding domain of TcdA (Gardiner *et al.*, 2009). A synthetic gene was created encoding the receptor-binding domain of TcdA and optimized for expression in human cells. Parenteral administration of this DNA vaccine induced neutralizing antibodies to CDA and protected mice from death.

The need to target both toxin A and B for effective immunotherapy

Many of the animal studies reviewed above have demonstrated that immunization against both toxins A and B, rather than against toxin A or toxin B alone, affords the best protection against CDI (Babcock *et al.*, 2006; Fernie *et al.*, 1983; Kim *et al.*, 1984; Kink & Williams, 1998; Libby *et al.*, 1982). Several investigators have focused on inducing protection against *C. difficile* toxin A alone because previous animal models had suggested that toxin B, although a potent cytotoxin, was not enterotoxic in animals (von Eichel-Streiber *et al.*, 1996). In the hamster and murine model, toxin A, but not toxin B, was lethal when administered intragastrically (Lyerly *et al.*, 1985). Purified toxin B did not cause any symptoms of disease unless intestinal damage was present or the toxin was co-administered with toxin A.

More recently there has been a paradigm shift in the understanding of the role of toxin B in CDI. Using isogenic *tcdA* and *tcdB* mutants of *C. difficile*, investigators have shown that both toxin A and toxin B contribute to disease pathogenesis in animal models (Kuehne *et al.*, 2010; Lyras *et al.*, 2009). Studies using human colon have shown that toxin B is a more potent enterotoxin than toxin A in human colon (Riegler *et al.*, 1995; Savidge *et al.*, 2003). Furthermore, human strains of toxin A-negative, toxin B-positive *C. difficile* have emerged which have been associated with sporadic and epidemic CDI (Drudy *et al.*, 2007). Taken together, these findings suggest that toxin B plays an important role in the pathogenesis of CDI, but do not disprove the role of toxin A in human disease. Thus, it is important that vaccines and passive antibody products that protect against and treat CDI in humans target both toxins.

Passive immunization against *C. difficile* toxins in humans

(i) Oral passive immunization. There have been few studies examining the role of oral administration of anti-*C. difficile* antibodies for the prevention or treatment of CDI in humans. Despite promising results in animals and in a small human study, anti-*C. difficile* BIC has not been developed further for human use (Kelly *et al.*, 1996; Warny *et al.*, 1999). Using a similar approach, van Dissel *et al.* (2005) developed an anti-*C. difficile* bovine immunoglobulin secretory IgA concentrate (40 % immune whey protein concentrate; Mucomilk) prepared from the milk of

cows immunized against *C. difficile* and its toxins. Following successful studies in hamsters, the investigators found in an uncontrolled pilot study that Mucomilk was safe and well-tolerated in 16 patients with CDI (van Dissel *et al.*, 2005). None of the patients treated experienced recurrent CDI. In a later randomized, double-blind study, Mucomilk was found to be as effective as metronidazole in reducing recurrent CDI (Mattila *et al.*, 2008). Unfortunately, this study was terminated early because of a lack of funding.

(ii) Intravenous passive immunization. Normal pooled immunoglobulin preparations contain substantial amounts of both IgG antitoxin A and B and demonstrate toxin neutralizing activity *in vitro*. In an early case series of five children with relapsing CDI and low antitoxin antibody levels, intravenous immunoglobulin (IVIG) treatment resulted in resolution of their symptoms in association with an increase in antitoxin antibody levels (Leung *et al.*, 1991). Since that time, IVIG has been used to treat many cases of severe or refractory CDI with mixed results (Abougergi *et al.*, 2010; O'Horo & Safdar, 2009; Wilcox, 2004). Different doses of IVIG (150–400 mg kg⁻¹) administered at varying frequencies (one to three doses), with or without adjunctive treatment with oral anti-*C. difficile* antibiotics, have produced varying results. Despite these many case reports and observational studies, there have been no randomized controlled clinical trials of IVIG for CDI. Likewise, there is no standardized algorithm for its use. Most recently, Abougergi *et al.* (2010) reported a high mortality rate (57%) in 21 patients treated with IVIG for severe CDI. The authors found that higher Acute Physiology and Chronic Health Evaluation II (APACHE II) scores on the day of IVIG infusion were significantly associated with higher mortality. They suggest that IVIG may have a role to play in patients with severe CDI that is confined to the colon but that once extracolonic organ dysfunction and systemic inflammatory response syndrome develop it may be less beneficial. This is in keeping with our earlier observational studies of the immune response to *C. difficile* in humans, in which we also found that critically ill patients were less likely to be protected against symptomatic CDI despite having high antitoxin antibody levels compared to less severely ill patients with similar antibody levels (Kyne *et al.*, 2000).

A recent major advance in passive immunotherapy for CDI has been the development of HumAbs against *C. difficile* toxins A and B for use in humans (Leav *et al.*, 2010; Lowy *et al.*, 2010). Following demonstration of efficacy in hamsters and safety in a phase I study in healthy volunteers, these HumAbs were tested in a large multi-centre, randomized, double-blind, placebo-controlled trial in which the primary end point was recurrence of CDI (Babcock *et al.*, 2006; Leav *et al.*, 2010; Lowy *et al.*, 2010). The HumAbs were administered intravenously as an adjuvant to standard antimicrobial therapy in patients with symptomatic CDI. HumAb treatment was associated

with a 72% relative reduction in recurrence rates compared to placebo but did not reduce the severity of infection or the duration of diarrhoea or hospitalization (Lowy *et al.*, 2010). Patients with multiple recurrences had an 82% relative reduction in recurrence compared to the placebo group. The anti-*C. difficile* HumAbs were as safe and as well-tolerated as placebo and had circulating half-lives up to 26 days. Future studies are needed to determine whether monoclonal antibodies will be cost-effective for the treatment of initial cases of CDI. They may also be useful in patients with refractory or severe CDI or may be beneficial as prophylaxis in very high risk patients such as those with prolonged hospitalizations or in intensive care unit (ICU) settings.

These recent human studies on the use of HumAbs against *C. difficile* toxins are important for several reasons. First, they lend strong support to a cause and effect association between high serum antitoxin concentrations and protection against CDI. Second, they demonstrate the protective efficacy of passive immunization. Finally, they confirm the role of serum IgG antitoxin in protection against an infection that is predominantly confined to the large intestine. The mechanism by which serum antibodies protect against toxin-mediated disease in the colon is still not well understood. Antitoxin antibodies may be exuded through an inflamed colonic mucosa, as both toxins A and B increase the permeability of intestinal epithelial cells by disrupting tight junctions (Giannasca *et al.*, 1999; Riegler *et al.*, 1995). An alternative mechanism is the active transport of antitoxin antibodies via the FcRn receptor, which mediates bidirectional transport of IgG across the intestinal epithelium (Yoshida *et al.*, 2006).

Several studies have suggested a role for secretory IgA in protection against *C. difficile* and its toxins (Kelly *et al.*, 1992; Stubbe *et al.*, 2000; Wada *et al.*, 1980; Ward *et al.*, 1999; Warny *et al.*, 1994). However, the importance of secretory IgA versus systemic IgG responses is unclear and recent studies indicate that systemic IgG responses may be more important in determining clinical outcomes in CDI (Kyne *et al.*, 2000, 2001; Lowy *et al.*, 2010).

Active immunization with a *C. difficile* toxoid vaccine in humans

The first candidate vaccine against *C. difficile* that has been tested in humans is a toxoid vaccine containing formalin-inactivated purified toxins A and B. In healthy volunteers, intramuscular administration of this vaccine was found to be safe, well-tolerated and associated with serum antitoxin antibody levels significantly higher than those observed in natural infection (Aboudola *et al.*, 2003; Kotloff *et al.*, 2001). Vaccination was also successful in treating a small number of patients with recurrent CDI (Sougioultzis *et al.*, 2005). Sanofi-Aventis are currently sponsoring phase II clinical trials of their *C. difficile* toxoid vaccine for the prevention of CDI (clinicaltrials.gov NCT00772343 and NCT01230957).

Lee *et al.* (2010) recently published an economic computer simulation model of the potential value of a *C. difficile* toxoid vaccine. They found that over a wide range of vaccine costs, vaccine efficacies and *C. difficile* risks the vaccine would be cost-effective for the prevention of CDI in high-risk patients and also for the prevention of recurrent CDI. In particular, they noted that, as *C. difficile* risk and vaccine efficacy increased, vaccination became economically dominant, having the potential to save society, third-party payers and hospitals money, while preventing morbidity and mortality.

Future vaccines to reduce or prevent colonization with *C. difficile*

As a *C. difficile* toxoid vaccine is unlikely to affect colonization, alternative vaccine candidates are being explored that may reduce or prevent this first step in the pathogenesis of CDI (Brun *et al.*, 2008; Ní Eidhin *et al.*, 2008; Péchiné *et al.*, 2007). Péchiné *et al.* (2005a, b) have evaluated *C. difficile* surface proteins, including the flagellar cap protein FliD and Cwp84, as vaccine antigens in a human flora-associated mouse model. After rectal immunization with different antigen combinations and subsequent toxigenic *C. difficile* challenge, colonization levels of *C. difficile* were significantly lower in mice who received the antigen combinations than in mice who received control PBS. However, immunization did not prevent *C. difficile* colonization. Similarly, Ní Eidhin *et al.* (2008) found no significant benefit of active immunization of hamsters using SLP.

Future multi-component vaccines may be developed which incorporate both neutralization of toxins and prevention or reduction in colonization by *C. difficile* through targeting surface proteins or other factors needed for colonization and persistence of infection. Preventing colonization may have the added benefit of reducing bacterial carriage, environmental contamination and spread thereby augmenting the beneficial effects of herd immunity to *C. difficile*.

Innate immune responses in CDI

The intracellular mechanism of action of *C. difficile* toxins A and B is to catalyse the glucosylation of a conserved threonine residue on specific Rho family members (Just *et al.*, 1995). This leads to both activation and inactivation of Rho signalling and ultimately to cell death and a pronounced host inflammatory response (Genth *et al.*, 2008; Sun *et al.*, 2009). Pathogenic, toxin A-negative strains of *C. difficile* produce toxin B variants with atypical cytotoxic effects including cell rounding and the development of filopodia-like structures that have been linked to glucosylation of R-Ras and RhoA activation, respectively (Chaves-Olarte *et al.*, 2003). It has been suggested that differences in the intracellular effects of toxin B variants produced by toxin A-negative strains (compared to toxin B

from toxin A-positive strains) may be responsible for the ability of these strains to cause disease in humans. Innate inflammatory responses that are activated by the glucosyl-transferase activity of *C. difficile* toxins A and B include the NF- κ B and MAP kinase pathways (Chae *et al.*, 2006; Chen *et al.*, 2006; Jefferson *et al.*, 1999; Kim *et al.*, 2005; Warny *et al.*, 2000). Monocytes and macrophages are activated and a variety of proinflammatory cytokines including IL-1 β , TNF- α and IL-8 are released (Jefferson *et al.*, 1999; Linevsky *et al.*, 1997; Sun *et al.*, 2009; Warny *et al.*, 2000).

The intense acute inflammatory response appears to be a major factor in causing intestinal injury in CDI (Savidge *et al.*, 2003). Acute colitis with pseudomembrane formation and a heavy neutrophil infiltration of the colonic epithelium and submucosa is characteristic of severe CDI. Furthermore, as discussed below, a marked increase in neutrophils in the peripheral blood is also a feature of severe CDI and is linked to prognosis (Cloud *et al.*, 2009; Lamontagne *et al.*, 2007; Loo *et al.*, 2005; Pépin *et al.*, 2007, 2009). In one study, neutrophil infiltration of the intestine was prevented in rabbits by using a monoclonal antibody to block CD18, a neutrophil cell surface receptor that mediates firm adhesion to the vascular endothelium, an essential step in neutrophil diapedesis (Kelly *et al.*, 1994). Exposure to *C. difficile* toxin A caused severe inflammation and tissue destruction of control intestine whereas anti-CD18 pretreatment led to almost complete protection. Interestingly, mild changes remained in the intestinal epithelium of anti-CD18-treated animals with increased numbers of apoptotic epithelial cells. One explanation for these findings is that *C. difficile* toxins cause direct injury to the intestinal epithelium, which is associated with a robust host inflammatory response with neutrophil activation and recruitment leading in turn to greatly amplified intestinal injury. In fact, a wide range of anti-inflammatory agents can reduce intestinal injury in animal models of CDI (Anton *et al.*, 2004; Chen *et al.*, 2006; Cottrell *et al.*, 2007; Kim *et al.*, 2005, 2007; Kokkotou *et al.*, 2009; Pothoulakis *et al.*, 1993; Warny *et al.*, 2000). The use of combinations of antibiotic and anti-inflammatory agents to treat severe CDI in humans warrants greater attention and investigation, especially given the rising incidence of severe and fatal disease (McDonald *et al.*, 2006; Redelings *et al.*, 2007).

Clinical prediction tools for CDI: using host factors to predict disease outcomes

As discussed above, there is a spectrum of host responses to CDI, ranging from symptomless carriage to fulminant and fatal colitis (Kelly & LaMont, 2008). As the treatment options for CDI expand to include probiotic therapy (possibly including non-toxinogenic *C. difficile*), newer antibiotics with lower recurrence rates, and immune-based therapies including passive antitoxin infusion and active vaccination, it becomes increasingly important to identify those individuals at greatest risk for recurrent, severe or fatal disease (O'Donoghue & Kyne, 2010; Venuto *et al.*,

2010). In that way, new treatment approaches can be applied to greatest effect and at the lowest cost-to-benefit ratio. Thus, there is renewed interest in the development and validation of clinical prediction tools for CDI, both to guide clinical practice and to aid clinical research and drug development.

Predicting primary CDI

The single greatest clinical problem in CDI is the recent dramatic and sustained increase in disease incidence, which is now three times that seen in 2000 both in the US and in many parts of Europe (McDonald *et al.*, 2006; Redelings *et al.*, 2007). A method to reliably identify those patients at greatest risk for CDI will be valuable in applying prophylactic measures in the form of: (i) more sparing antibiotic use, (ii) use of antibiotics that are associated with lower risk for CDI, (iii) targeted and more extensive measures to reduce exposure to *C. difficile* and its spores and (iv) use of novel probiotics or other novel agents to protect against CDI (Cohen *et al.*, 2010; Crobach *et al.*, 2009). Identification of high-risk patients can also facilitate the early diagnosis and treatment of CDI when it occurs.

Risk factors for CDI have been described in many studies and include antibiotic use, admission to a health-care facility, prolonged and multiple antibiotic use, prolonged hospital stay, advanced age, increased co-morbidities, gastrointestinal surgery, nasogastric intubation and more (Cohen *et al.*, 2010; Howell *et al.*, 2010; Kelly & LaMont, 2008). However, few studies have attempted to predict the risk for CDI based on combinations of these individual risk factors. We described previously that individuals who receive antibiotics while in hospital are at increased risk for CDI if they have critical underlying disease (Kyne *et al.*, 2002). In this study of elderly, hospitalized antibiotic recipients, those with severe or extremely severe underlying disease (modified Horn's Index of 3 or 4) had incidence rates of CDI of 8.7 % in the derivation cohort and 11 % in the validation cohort (Kyne *et al.*, 2002). It is probable that clinical prediction algorithms that utilize a wider variety of risk factors can be more accurate in disease prediction but the development and testing of such complex prediction tools will require studies in very large at-risk populations.

Predicting severe CDI

In a landmark study, Zar *et al.* (2007) compared treatment of CDI for 10 days with oral metronidazole 250 mg four times daily to oral vancomycin 125 mg four times daily. Patients were prospectively stratified as having mild or severe CDI based on clinical criteria [severe defined as two points or greater; one point each for age over 60 years, temperature above 38.3 °C (101 °F), serum albumin of less than 2.5 mg dl⁻¹, peripheral white blood cell count above 15 000 cells mm⁻³; two points for pseudomembranous colitis at colonoscopy or requirement for ICU care]. In patients with mild CDI, responses to metronidazole (90 %) and to vancomycin (98 %) were similar ($P=0.36$). In severe

CDI, response rates to metronidazole (76 %) were significantly lower than to vancomycin (97 %, $P=0.02$). These study findings indicate that vancomycin is more effective than metronidazole in severe CDI and have led to recommendations that oral vancomycin be used as first-line therapy in severe CDI (Cohen *et al.*, 2010).

Although the study by Zar *et al.* (2007) showed that clinical criteria can predict response to therapy in CDI, the scoring system used was not formally evaluated or validated. Other investigators have also identified several clinical parameters that are associated with poor clinical outcomes in CDI, such as a requirement for ICU admission, colectomy or death (Cloud *et al.*, 2009; Lamontagne *et al.*, 2007; Loo *et al.*, 2005; Pépin *et al.*, 2004, 2007, 2009). Several studies have identified an elevated peripheral white blood cell count and a rising serum creatinine concentration as important predictors of poor outcomes in CDI (Cloud *et al.*, 2009; Lamontagne *et al.*, 2007; Loo *et al.*, 2005; Pépin *et al.*, 2004, 2007, 2009). Additional prospective studies are needed to develop and validate clinical prediction tools for severe CDI in order to guide choice of therapy and optimal timing of surgery (Cohen *et al.*, 2010).

Predicting recurrent CDI

Recurrent CDI complicates 15–25 % of CDI cases. A number of risk factors for recurrent CDI have been identified. A recent meta-analysis highlighted the continued use of antibiotics other than those used to treat CDI (OR: 4.23; 95 % CI: 2.10–8.55; $P<0.001$), use of acid anti-secretory medications (OR: 2.15; 95 % CI: 1.13–4.08; $P=0.019$) and older age (OR: 1.62; 95 % CI: 1.11–2.36; $P=0.0012$) as factors that were significantly associated with increased risk of recurrent CDI (Garey *et al.*, 2008).

We recently developed and validated a prediction tool for recurrent CDI based on three clinical factors: (i) age greater than 65 years, (ii) severe or extremely severe underlying disease (modified Horn's Index 3 or 4) and (iii) continued antibiotic use (i.e. other than metronidazole or vancomycin) after CDI therapy (Hu *et al.*, 2009). The clinical prediction rule discriminated between patients with and without recurrent CDI, with an area under the curve of the receiver operating characteristic curve of 0.80 (95 % CI: 0.67–0.92) in the validation cohort. The rule correctly classified 72 % of patients as having a single episode of CDI versus recurrence. Thus, a relatively simple and validated tool that is based on readily available clinical parameters can be used to predict recurrent CDI and to identify high-risk patients most likely to benefit from measures to prevent recurrence.

In another study, we found that serum IgG antibodies against *C. difficile* toxins A and B were higher in subjects with a single episode of CDI than in those with recurrent disease (Kyne *et al.*, 2001). However, measuring serum antitoxin concentrations added little to the clinical parameters listed above in predicting recurrent CDI (Hu *et al.*, 2009).

Conclusions

There are several notable unmet medical needs in CDI. The first and most fundamental is the alarming increase in the number of cases reported in recent years. The second is the marked worsening of disease severity which, compounded to the increased incidence, has led to sharp rises in CDI-related mortality. The third is the ever-present problem of recurrence. Adaptive immune responses, especially against *C. difficile* toxins A and B, are now known to influence disease expression and course. Both active vaccination and passive immunotherapy approaches to CDI prevention and treatment show substantial clinical promise. Conversely, innate immune responses to CDI appear to be counter-productive and to exacerbate toxin-mediated intestinal injury. Agents to temper the innate immune response, used in conjunction with antibiotics, hold the potential to improve clinical outcomes in severe disease. Validated clinical prediction tools, based on host characteristics, will be useful to identify those at greatest risk for complications of CDI and therefore the best candidates for current and future prophylactic and therapeutic interventions.

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