Prospects for a Vaccine for
Clostridium difficile

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Abstract

Clostridium difficile diarrhoea and colitis is a new disease that is attributable to broad spectrum antibiotic therapy. During the past 2 decades *C. difficile* has become one of the most common nosocomial pathogens in the developed world. As changing demographics create an increasingly elderly population and the use of broad spectrum antimicrobials continues to expand, *C. difficile* is likely to become increasingly problematic.

Disease caused by this organism is caused by the inflammatory actions of its 2 toxins, A and B, on the intestinal mucosa. Human antibody responses to these toxins are common in the general population and in patients with *C. difficile*-associated disease. There is substantial, albeit inconclusive, evidence to indicate that antitoxin antibodies provide protection against severe, prolonged or recurrent *C. difficile* diarrhoea.

Immunity induced by oral or parenteral passive administration of antibody is protective in animal models of *C. difficile* infection. In humans, intravenous passive immunisation with pooled human immunoglobulin has been successful in the treatment of recurrent and severe *C. difficile* colitis. Human trials of oral passive immunotherapy with bovine immunoglobulin therapy are in progress. Formalin–inactivated culture filtrate from toxigenic *C. difficile*, as well as purified and inactivated toxins, have been used to successfully immunise animals. Similar preparations are under investigation as possible human vaccines.

Antibiotic therapy is effective in treating most individual patients with *C. difficile* diarrhoea, but has proven ineffective in reducing the overall incidence of nosocomial infection. Active immunisation is probably the most promising approach to long term control of this difficult iatrogenic disease.

1. *Clostridium difficile* Colitis: The Burden of Disease

Clostridium difficile is now the leading infectious cause of nosocomial diarrhoea in developed countries.[1] Epidemiological surveys have shown that 15 to 21% of in-patients are infected by this organism during the course of their hospital stay.[2,3] The exact prevalence of *C. difficile* colitis is not known but there are at least 500 000 and possibly as many as 3 million cases per annum in the US. Elderly, debilitated patients are particularly vulnerable and higher infection rates have been reported in this patient group. Residents of long term care facilities are also at risk; colonisation rates as high as 73% have been reported in some facili-

ties.[4] Although previously considered rare, the incidence of community-acquired *C. difficile* infection and colitis also appears to be increasing.[5,6]

The medical implications and costs of *C. difficile* infection are substantial. Severe disease is associated with significant morbidity and mortality. Patients may require intensive care unit admission, prolonged therapy with oral and intravenous antibiotics or surgery. The mortality rate for severe *C. difficile* colitis has been reported to be as high as 65%.[7,8] Even mild *C. difficile* diarrhoea is costly, since it will prolong hospital stay by 1 to 2 weeks.[9-11] Crude estimates for the cost of infection range from £4000 per case in the UK to $US12 000 per case in the US in 1996.[9,10] Hospital outbreaks of *C. difficile*–associated disease (CDAD) occur frequently and are associated with substantial patient morbidity and mortality as well as considerable financial cost.[12-14]

2. Conventional Management of *C. difficile* Colitis

Conventional therapy of CDAD involves discontinuation of the inciting antimicrobial and supportive therapy.[15,16] Approximately 23% of patients will respond to this approach with resolution of symptoms within 48 to 72 hours.[17] If symptoms are prolonged, therapy with oral metronidazole or vancomycin is indicated. Although metronidazole is recommended as the first line agent, the choice of therapy depends on disease severity, antimicrobial tolerance and response.[15,16] While antibiotic therapy is often successful in treating initial episodes of diarrhoea, these agents are of no benefit in the treatment of asymptomatic carriers and are unsuccessful in eradicating spores, which are the main transmissible form of this organism.[15,16] In addition, 20% of patients treated with metronidazole or vancomycin will relapse when treatment is discontinued and many of these will have multiple relapses.[16] Patients who experience 2 relapses have a 65% risk of further recurrence of *C. difficile* diarrhoea, making this form of CDAD a substantial management problem.[18,19]

The emergence of vancomycin resistance among organisms such as *Enterococcus faecalis* has limited the use of oral vancomycin.[20] Although metronidazole resistance in *C. difficile* isolates is currently rare, experience with *Helicobacter pylori* indicates that resistance may develop rapidly with increased use.[15,21]

The limitations of conventional therapy, the incongruity of treating antibiotic-induced diarrhoea with additional antibiotics and the alarming increase in the incidence of CDAD disease over the past 2 decades require the development of new strategies to limit the impact of this opportunistic pathogen. The medical community is keen to limit this infectious disease since most cases of *C. difficile* colitis are nosocomial and virtually all are iatrogenic.

3. Disease Pathogenesis

The sequence of events leading to *C. difficile* diarrhoea and colitis is outlined in figure 1.[22] Antimicrobial therapy causes a disruption of the normal colonic microflora predisposing to colonisation by *C. difficile*. Hospital patients receiving antibiotics are especially likely to be infected since *C. difficile* and its spores are prevalent in that environment. *C. difficile* is a non-invasive organism. Pathogenic strains produce 2 large (~300kD) protein exotoxins, toxin A and toxin B, which have cytotoxic, enterotoxic and pro-inflammatory effects resulting in diarrhoea and colitis (fig. 2, table I).[16,23]

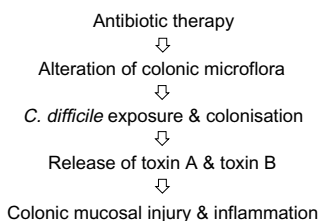**Pathogenesis of *Clostridium difficile* diarrhoea and colitis**

Antibiotic therapy
⇩
Alteration of colonic microflora
⇩
C. difficile exposure & colonisation
⇩
Release of toxin A & toxin B
⇩
Colonic mucosal injury & inflammation

Fig. 1. Pathogenesis of *Clostridium difficile*-associated diarrhoea and colitis (after Linevsky & Kelly,[22] with permission).

Fig. 2. Resected colon from a patient with pseudomembranous colitis (severe *Clostridium difficle* colitis). Typical pseudo-membrane plaques are visible on the colonic mucosa. The surrounding mucosa is hyperemic but not ulcerated (reproduced from Kelly et al.,[16] with permission).

Toxins A and B are encoded by 2 distinct genes positioned in close proximity on the bacterial genome.[24-26] They are structurally similar and show 49% homology at the amino acid level.[25] Both toxins carry repeated peptide units towards their carboxyl terminus which are believed to mediate receptor binding.[26] Both toxins also share a common intracellular mechanism of action, acting as enzymes to catalyse the glucosylation of a threonine residue on Rho family, guanosine triphosphate (GTP)–binding proteins.[27,28] Rho glucosylation results in disruption of the actin cytoskeleton, cell rounding and cell death. Both toxins also stimulate release of cytokines and other inflammatory mediators from intestinal inflammatory cells and activation of the enteric nervous system.[29-33]

4. Immune Response to *C. difficile* Infection

Since *C. difficile* diarrhoea is toxin-induced, an effective antitoxin antibody response may be protective. In fact there is already considerable evidence, as presented below, that immunisation against *C. difficile* toxins may be a highly effective approach to controlling CDAD.[33]

4.1 Prevalence of Serum and Mucosal Antibodies in the General Population

Low levels of serum IgG and IgA to *C. difficile* toxins can be demonstrated in most healthy children and adults.[34-37] This probably indicates prior exposure to toxigenic *C. difficile*. The prevalence of antibodies increases after the first 2 years of life and appears to decline in old age.[34,38,39]

Mucosal secretory IgA responses are also common.[35,36] Human colonic aspirates frequently contain secretory IgA antibodies to toxin A and these antibodies inhibit binding of toxin to its intestinal receptors.[35] Secretory IgA with neutralising activity against *C. difficile* toxins has also been demonstrated in normal human colostrum.[35,40,41]

4.2 Prevalence of Serum and Mucosal Antibodies in *C. difficile*–Associated Disease

The relationship between the host immune response and the clinical course of infection is not clearly defined. Patients with CDAD develop antitoxin antibody responses which may represent a primary response to infection or the boosting of an

Table I. Comparison of *Clostridium difficile* toxins (after Pothoulakis,[23] with permission)

| Property	Toxin A	Toxin B
Molecular weight	308 000kD	270 000kD
Effects in animal ileal loops *in vivo*	Fluid secretion, inflammation Increased permeability Epithelial cell necrosis Activation of neuroimmune cells	None
Effects on human colonocytes *in vitro*	Epithelial cell damage and increased mucosal permeability	Epithelial cell damage and increased mucosal permeability
Cellular effects	Inactivation of Rho Actin disaggregation Cell rounding	Inactivation of Rho Actin disaggregation Cell rounding
Receptors	Present on animal enterocytes Absent on newborn rabbit intestine Present on human colonocytes	Absent on animal enterocytes Present on human colonocytes
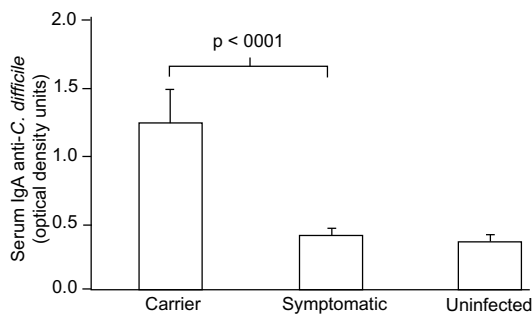
Fig. 3. Serum IgA antibodies to *Clostridium difficile* in asymptomatic carriers (n = 5), patients with symptomatic *C. difficile* infection (n = 21) and healthy individuals (n = 26). Data expressed as mean optical density ± SE. (reproduced from Kelly,[33] with permission).

anamnestic immune response.[36,37,39] Based on the observation that most patients colonised by toxigenic *C. difficile* are asymptomatic, it is tempting to postulate that the human immune response may 'protect' these individuals from enteric disease. The evidence to support this is substantial but not conclusive. Mulligan et al.[42] demonstrated higher serum levels of IgA and IgM antibodies against *C. difficile* somatic cell antigens in asymptomatic carriers compared to patients with CDAD (fig. 3). In contrast, Johnson et al.[37] found that patients with asymptomatic infection had lower mean serum IgG and intestinal IgA antitoxin antibody levels than symptomatic patients.

In symptomatic patients, an effective antitoxin antibody response in some patients appears to protect against severe or recurrent *C. difficile* diarrhoea and colitis. Aronsson et al.[43] reported that a lack of serum IgG antibodies to *C. difficile* toxin B correlated with more severe colitis and that high antitoxin B IgG titres correlated with clinical recovery without subsequent relapse.[44] Warny et al.[36] also found that serum IgG and faecal IgA antitoxin A levels were significantly higher in patients who experienced a brief, single episode of diarrhoea compared to those with prolonged or relapsing CDAD (fig. 4). In a study of children with recurrent *C. difficile* diarrhoea, lower A levels

were found in affected children compared with healthy children or adults.[45]

5. Passive Immunisation of Animals and Humans against *C. difficile* Toxins

Kim et al.[46] have shown that hamsters immunised against *C. difficile* toxins can confer protection to suckling hamsters via their breast milk. Parenteral passive immunotherapy is also effective in animals.[47,48] In one study, Corthier et al.[48] administered a monoclonal antibody against the putative binding region of toxin A to axenic mice. The treated animals survived subsequent challenge with a toxigenic strain of *C. difficile* but control mice died within 2 days of this challenge.[48] In protected mice, faecal levels of toxin B were similar to those in dying mice, but faecal toxin A levels were greatly reduced. This study demonstrates the ability of systemic antitoxin A antibodies to protect against toxin-induced intestinal disease and, along with other studies, indicates that in animals toxin A is a more important enterotoxin than toxin B.[49,50]

We have used passive immunotherapy with intravenous immunoglobulin in a small number of patients with recurrent or severe *C. difficile* diarrhoea.[45,51] As indicated in section 4.1, most healthy adults have serum IgG antibodies against *C. difficile* toxins.[34,35,38] As a result, normal pooled immunoglobulin preparations contain substantial amounts of both IgG A and B antitoxins and demonstrate toxin neutralising activity.[51] Immunoglobulin infusion was used successfully to treat relapsing CDAD in 5 children with low antitoxin antibody levels.[45] Pooled human gamma globulin infusion, caused a marked increase in their serum antitoxin antibody levels (fig. 5) and was associated with resolution of *C. difficile* toxin-induced diarrhoea.[45] Intravenous immunoglobulin therapy may also be effective in treating patients with severe *C. difficile* colitis who do not respond to standard therapy with metronidazole and vancomycin.[51] Work is under way to immunise volunteers and produce human hyperimmune globulin against *C. difficile* toxins (Thomas Monath, per-
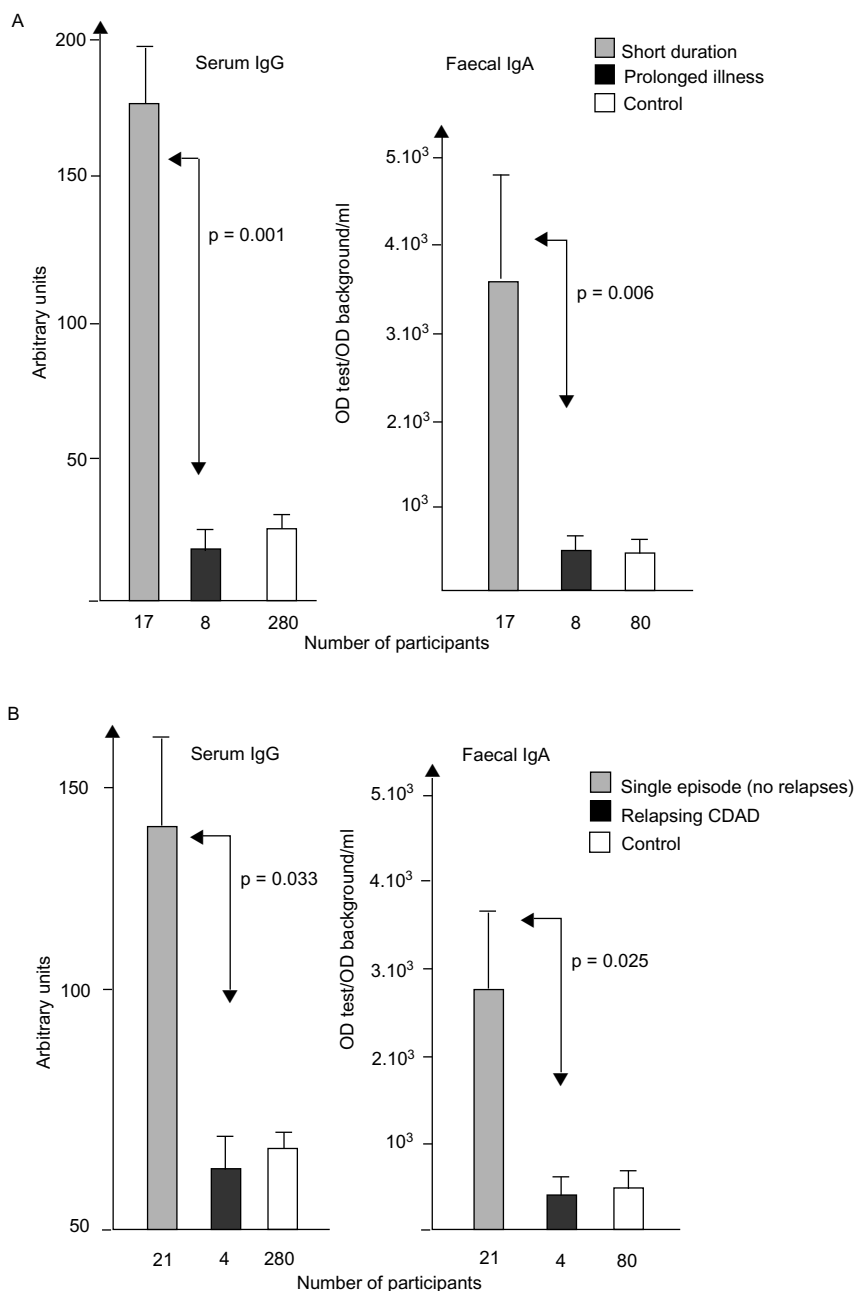
Fig. 4. Antitoxin A serum IgG and faecal IgA levels in nonimmunocompromised patients and controls. **A** Relation of antibody levels to the time duration of CDAD. Eight patients with prolonged CDAD (>2 weeks) including 4 with relapses, had serum and faecal titres significantly lower than those with symptoms of shorter duration. **B** Relation of antibody levels to the presence of relapses. Serum IgG and faecal IgA antitoxin A levels were significantly higher in patients who presented a single episode (n = 21) than in those with relapsing CDAD (n = 2), who had titres at control levels (reproduced from Warny et al.,[36] with permission). **CDAD**= *Clostridium difficile*-associated disease; **OD** = optical density.

sonal communication). If successful, this will open the way for studies on the efficacy of passive parenteral immunotherapy for CDAD in humans.

Human trials of oral passive immunotherapy are already under way.[52-54] These studies use bovine immunoglobulin concentrate (BIC) produced from the colostrum of cows immunised against *C. difficile* toxins. BIC–*C. difficile* contains high levels of neutralising antibodies against toxins A and B and protects against *C. difficile* enterocolitis in animal models.[52,55] The efficacy of BIC–*C. difficile* in treating *C. difficile* diarrhoea is currently being tested in clinical trials.

6. Active Immunisation against *C. difficile* and its Toxins

6.1 Vaccine Antigens in Animal Studies

Since *C. difficile* colitis is toxin-induced, toxins A and B are the primary vaccine targets. Nontoxin antigens have not been extensively evaluated as there is no evidence to suggest that immunity to cell wall or other bacterial factors is important in protecting against disease.

In animal models, toxin A is a potent enterotoxin and appears to be the main cause of intestinal injury and inflammation.[48-50] Immunisation against toxin A alone has been protective in some animal studies.[46,48,56] However, toxin B is a more potent cytotoxin *in vitro* and may work in synergy with toxin A to exacerbate intestinal injury in animals. Libby et al.[57] reported that all hamsters survived clindamycin (known to be a cause of CDAD) challenge when immunised against both toxins, while only 24% of hamsters immunised against either toxin A or B survived. Fernie et al.[58] also concluded that immunisation against both toxins afforded the highest degree of protection. In their studies, immunisation with toxoid A gave no protection, while toxoid B gave 30% protection and immunisation against both toxins protected ≥80% of hamsters.[58] Recent human studies indicate that toxin B is more potent than toxin A in causing injury to the human colon.[59] Thus, in humans, an
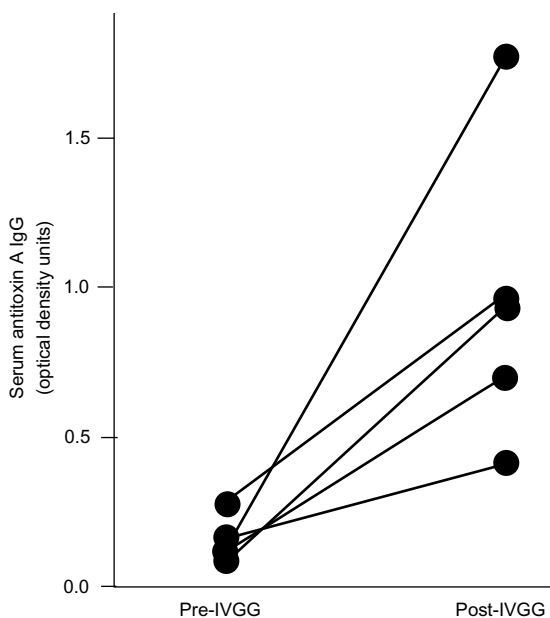

Fig. 5. Serum IgG antibody levels to *Clostridium difficile* toxin A are shown for each of 5 children with long term relapsing *C. difficile* colitis before and after intravenous gamma globulin (IVGG) therapy. Therapy with IVGG resulted in a rise in antitoxin A IgG in all patients (p = 0.01) [reproduced from Leung et al.,[45] with permission].

effective immune response would be likely to require neutralisation of both toxin A and toxin B.

Partially purified, formalin–inactivated culture filtrates have been used in a number of animal studies and have shown protective immunity.[58,60] Other investigators have examined more purified antigen preparations as potential vaccines. Partially purified toxoid A and B have been used for immunisation, either singly or in unison.[46,57,58] More recently, the nontoxic repeated binding sequence of toxin A has been expressed as a fusion protein in an attenuated *Vibrio cholera* vector strain and used for oral immunisation in rabbits.[61] However, culture filtrates from a highly toxigenic strain of *C. difficile* continue to be the most attractive source of toxin antigen. They are relatively inexpensive to produce and have demonstrated efficacy in animal studies. Highly purified or recombinant antigens are more expensive; a multivalent

vaccine is likely to be required to effectively neutralise both toxins, and the key epitopes for protective immunity have not been adequately defined as yet.

6.2 Route of Vaccine Administration

The route of vaccine administration is also likely to be an important determinant in eliciting protective immunity. All of the earlier animal work focused on protective immunity induced by parenteral immunisation. Recent studies by Torres et al.[60] in hamsters demonstrated success with a regimen involving a combination of parenteral and mucosal (intranasal) immunisation.[60] In that study, parenteral immunisation protected against death, but provided only 40% protection against diarrhoea. In contrast, animals who received intranasal immunisation followed by booster parenteral immunisation acquired 100% protection against both diarrhoea and death from *C. difficile*–related causes.[60]

In humans, experience with other enteric pathogens such as *V. cholera* has emphasised the importance of secretory IgA antibodies and mucosal memory for protection against disease.[61] An oral B subunit–whole cell (B-WC) vaccine has proved to be well tolerated and to provide long term protection against cholera in extensive trials, including a large field trial.[62] However, cholera toxin is a non-inflammatory enterotoxin whereas *C. difficile* toxins disrupt epithelial integrity and produce a marked intestinal inflammatory response.[23] This may allow transudation of neutralising serum antibodies into the intestinal mucosa and lumen.[45,48,51,60] It is, therefore, unclear whether mucosal immunisation will be a required element in protecting against *C. difficile* diarrhoea.

6.3 Who Should be Immunised?

A candidate human vaccine based on formalin-inactivated *C. difficile* toxoids has been prepared and tested both *in vitro* and in animal studies.[60] Initial clinical studies to determine tolerability and immunogenicity are planned. If a well tolerated and effective human vaccine can be developed, immunisation of high-risk individuals will be the first priority. These include elderly or debilitated individuals who are the most likely to receive antibiotics in a hospital setting and therefore most likely to experience *C. difficile* diarrhoea. Immunisation of nursing home residents should also be considered. These populations are similar to those currently targeted for influenza and pneumococcal immunisation. [64-66] Combining *C. difficile* toxoid immunisation with other established immunisations may facilitate an effective and inexpensive immunisation programme. The optimal route and scheduling of vaccine doses will need careful evaluation in the sick and the elderly, especially since systemic and mucosal immune responses to primary immunisation are compromised by aging.[67,68]

In the setting of hospitals and nursing homes, herd immunity is especially important in controlling endemic and epidemic infectious disease.[69] It is unlikely that a toxoid vaccine will directly affect *C. difficile* colonisation.[46,60] However, there may be a substantial indirect effect on colonisation rates if infected individuals are protected from intestinal disease, since patients with diarrhoea are a rich source of environmental contamination by *C. difficile* and the person-to-person spread of infection.[13,70,71]

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