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REVIEW

Intravenous Immunoglobulin for the Treatment of *Clostridium difficile* Infection: A Review

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Abstract *Clostridium difficile* infection (CDI) has increased sharply in incidence, mortality rate, and burden on the healthcare system over the past decade. Therefore, novel treatment modalities have been developed, including intravenous immunoglobulin (IVIG). The level of immune response to *Clostridium difficile* colonization is the major determinant of the magnitude and duration of clinical manifestations. This effect is mediated predominantly by serum IgG anti-toxin A antibodies. Based on this finding, anti-toxin A and B antibodies were successfully used in multiple in vitro and in vivo experimental settings to passively immunize hamsters in CDI models. In humans, IVIG was used as the source of those antibodies. Fifteen small, mostly retrospective and non-randomized reports documented IVIG's success in the treatment of protracted, recurrent, or severe CDI. Diarrhea resolution rates were higher in the former patient group, but the recurrence rates were similar. IVIG mechanism of action is neutralization of mainly toxin A through IgG anti-toxin A antibodies. Purified anti-toxin A and B antibodies were successfully used to decrease CDI recurrence rates among patients with no or one previous CDI episodes. In conclusion, the efficacy of IVIG for CDI treatment in animal models has been convincingly demonstrated. However, only few small non-randomized, mostly uncontrolled reports have been published on human subjects. A phase II trial results support the use of purified anti-toxin A and B antibodies to decrease CDI recurrence rates. Therefore, IVIG should currently only be used as adjunct therapy until results from large, randomized controlled trials are available.

Keywords *Clostridium difficile*/drug effects · Immunoglobulins · Intravenous/therapeutic use · Infectious diarrhea · Nosocomial infection · Immunomodulation

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Introduction

Clostridium difficile (*C. difficile*) infection (CDI) is the most common infectious cause of health care-acquired diarrhea [1]. Studies have estimated CDI direct cost as $1.1 billion annually across the United States [2], with each CDI episode costing an additional $3,500–$5,042 per occurrence [3]. Despite increasing awareness among health care professionals, the incidence, associated morbidity and mortality [4–6], and burden on health care resources have increased sharply over the past decade [5, 7, 8]. A number of factors appear to be involved in this process, including the increasingly advanced age of the population, increasing patients co-morbid illnesses [9], metronidazole treatment failure [10], and the spread of the BI/NAP1 *C. difficile* strain.

Toxin A and toxin B are mediators of *C. difficile*-induced infection. Toxin A is a 308-kDa protein enterotoxin with weak cytotoxic activity [11]. Toxin B is a 279-kDa cytotoxin that possesses 1,000-fold more potent cytotoxic activity compared to toxin A. Toxin B is weakly enterotoxic and was originally believed to gain access to enterocytes through the effect of toxin A [11, 12]. More recent experimental data, however, demonstrated that toxin B is the major virulence factor in CDI pathogenesis

[13, 14]. Combined, toxin A and toxin B can cause a large spectrum of clinical presentations, ranging from mild diarrhea that resolves with the discontinuation of antibiotics to a fatal, fulminant colitis.

Several reports including a meta-analysis describing metronidazole treatment failure have recently been published [15–18]. In addition, BI/NAP1, which has been associated with an increased CDI mortality and incidence especially of severe disease requiring colectomy [4, 6], has now been detected in North America, Canada, and Europe [4, 6, 19]. These recent findings emphasize the importance of finding alternative treatments for CDI. One such novel treatment is intravenous immunoglobulin (IVIG). After the first report of its successful use in the treatment of refractory CDI in 1991, IVIG has been utilized off-label to treat both refractory and fulminant CDI despite the lack of large randomized controlled trials. The aim of this article is to review the evidence, both in basic science and clinical research, supporting the use of IVIG for CDI treatment as well its mechanism of action and its results in clinical practice thus far. We used PubMed, Web of Science, Scopus, and Excerpta Medica databases to search for publications in peer-reviewed journals. Search words were "Clostridium," "Difficile", and "Immunoglobulin."

Passive Immunization in Animal Models

The background for the use of IVIG in CDI treatment was provided using hamster models of CDI. Allo et al. [20] first reported that *Clostridium sordellii* anti-toxin neutralized *C. difficile* toxins in vitro and in a hamster CDI model. Passive immunization of hamsters with *Clostridium sordellii* anti-toxin before or 24 h after clindamycin administration was found to prevent colitis and death. Similar results were reported by Kim et al. [21]. In these studies, only anti-toxin A antibodies were found to be protective against CDI, whereas anti-toxin B antibodies were not. Passive immunization against CDI was also studied in this report through the transfer of protection from immunized female hamsters to their infants. Only infant hamsters from mothers immunized with toxoid A were protected against *C. difficile*-associated ileocecitis, while infant hamsters from mothers immunized with toxoid B were not. Neutralizing antibodies to toxins A and B were detected in maternal milk and foster-mothering experiments confirmed that maternal protection was transferred to infant hamsters through breast milk.

Later, Lyerly et al. [22] specifically used passive immunization (hyperimmune oral immunoglobulin from bovine origin) to treat CDI in hamsters. Only hamsters receiving immunoglobulin before the development of diarrhea were protected from fatal cecitis. Further, when immunoglobulin administration was stopped, the protected mice developed diarrhea and died. The authors concluded that passive immunization before diarrhea development was protective against CDI and they speculated, based on their results, that the mechanism of action was toxin neutralization rather than organism elimination. The same results were demonstrated by Kink et al. [23] with oral administration of avian anti-toxin A and anti-toxin B antibodies. They also found that anti-toxin A antibodies, but not anti-toxin B antibodies, were protective when used alone and that both anti-toxin antibodies were more protective then either alone. However, in that model, hamsters remained protected after discontinuation of treatment, even after several re-challenges with *C. difficile*.

Immunity and *Clostridium difficile* Infection

The majority of healthy children and adults express serum anti-toxin A antibodies, presumably from a previous symptomatic or asymptomatic exposure to *C. difficile*. Viscidi et al. [24] demonstrated that anti-toxin A antibodies were present in 64% of patients more than 2 years of age and anti-toxin B antibodies were present in 66% of patients older than 6 months of age. While Leung et al. [25] found anti-toxin A antibody levels to be higher in healthy adult controls compared to healthy children, these levels fall with increasing age [26]. This appears to coincide with the known increased vulnerability to CDI with age. Furthermore, anti-toxin antibodies increase after resolution of diarrhea [24, 27, 28], which also coincides with the known decreased incidence of re-infection after a CDI episode, providing evidence that disease development is associated with the host immunological response.

The level of immune response to *C. difficile* colonization was later shown to be a major determinant of the magnitude and duration of clinical manifestations. Johnson et al. [29] found *C. difficile* anti-toxin A titers to be the lowest in sera taken from healthy control patients, with progressively higher titers in acutely ill patients, convalescent patients and asymptomatic *C. difficile* carriers. These observations were expanded by Mulligan et al. [30] to the immunoglobulin (Ig) A and IgM class of anti-toxin A antibodies. Later, Bacon et al. [26] found that 60% of patients with acute primary CDI demonstrated antibodies to toxin B, while only 28% with relapsing CDI had demonstrable antibodies. The association between disease duration and anti-toxin A levels was confirmed by Warny et al. [31], who measured anti-toxin A antibodies in the sera of patients with different symptom duration and found that the disease duration was inversely correlated with anti-toxin A antibody titers. Afterwards, Kyne et al. [32] prospectively measured levels of IgA, IgM, and IgG anti-toxin A antibodies in asymptomatic carriers and symptomatic patients.

The adjusted odds ratio for diarrhea was 48 among patients who had a low serum level of anti-toxin A IgG antibodies compared to those with high titers. The same findings were true for recurrent compared to non-recurrent disease, with an adjusted odds ratio of 48 [33]. Later, Katchar et al. [34] found that it is the IgG2 and IgG3 subclasses of anti-toxin A IgG antibodies that were deficient in recurrent disease.

IVIG Mechanism of Action in *C. difficile* Infection Treatment

The first report describing IVIG use for CDI treatment in humans was published in 1991 [25]. Leung et al. used IVIG to treat five children suffering from CDI with multiple recurrences despite antibiotic use. The patients' T and B cells activities were tested. The T-cell function was intact. However, the patients' IgG anti-toxin A antibody levels were significantly lower than those of healthy children. The authors reasoned that passively immunizing the experimental group by transfusing anti-toxin A IgG antibodies using IVIG would help in their recovery. All children receiving IVIG had clinical resolution of diarrhea while on therapy.

Since then, multiple in vitro and in vivo experiments confirmed that IVIG neutralizes toxin A and toxin B. IVIG is formed by pooling immunoglobulin from several donors, the majority of whom express high anti-toxin A and anti-toxin B antibody serum titers. In addition, high anti-toxin A and anti-toxin B antibodies levels were present in both the IVIG preparation and the recipients after infusion [25, 35–37].

While some early reports indicated that anti-toxin B antibodies were the major determinants of protection against colitis [27], later reports correlated disease severity pathologically [38] and clinically [29, 39] with anti-toxin A levels. Anti-toxin B antibodies were later shown to play an adjunctive role in conferring immunity against CDI when added to anti-toxin A antibodies, but not to have any significant role on their own [40–42]. This is consistent with the results in animal models of passive immunity.

Initial studies performed to identify the specific immunoglobulin subtype responsible for modulating IVIG protective effect resulted in different findings. For example, while Kyne et al. [32] demonstrated that serum anti-toxin A IgG antibodies correlated with protection against CDI, toxin-neutralizing activity was shown to be present exclusively in the IgA class of antibodies in vivo and in vitro [29–31, 42] specifically IgA1 [43]. Similarly, Johal et al. [44] demonstrated that colonic IgA-producing cells and macrophages were reduced in colonic biopsies of patients with CDI, and these levels were even lower in patients who subsequently relapsed compared to those who had only a single CDI episode. However, analysis of IVIG preparations demonstrated high anti-toxin A IgG antibody levels, but undetectable anti-toxin A IgA antibodies [25]. Further, serum anti-toxin A IgA antibody levels were unchanged after IVIG infusion in all 5 patients described by Leung et al. [25]. Other reports demonstrated a role for anti-surface layer proteins (SLP) antibodies, the most abundant surface localized proteins expressed by *C. difficile*, in symptoms development. Although IgM, IgA and IgG anti-SLP antibody levels did not differ among CDI asymptomatic carriers, patients with symptomatic disease and healthy controls, IgM anti-SLP levels were higher in patients who did not experience relapse compared to those who relapsed [45]. In addition, passive immunization of hamsters with anti-SLP antibodies prolonged their survival, although it did not prevent death [46]. Further, anti-SLP antibody treatment resulted in increased *C. difficile* phagocytosis and elimination by neutrophils [46].

The role of IgG subtype of anti-toxin A and B antibodies in neutralizing toxin A was described by Babcock et al. [40] who prepared several IgG anti-toxin A and anti-toxin B human monoclonal antibodies. The combination of the three different monoclonal anti-toxin A antibodies used could neutralize toxin A activity in vitro and prevent disease in the hamster model in vivo. Later analysis revealed that each of the three antibodies recognized a different toxin A domain: one neutralized toxin A enzymatic activity, while the second prevented toxin A binding to its receptor on enterocytes and the third prevented toxin internalization after binding to the receptor. Another possible mechanism was increased toxin elimination by phagocytes once bound to the antibody.

Overall, it is currently considered that the predominant IVIG mechanism of action is through binding and neutralization of toxin A by IgG anti-toxin A antibodies. The mechanism of IgG anti-toxin A antibody delivery to the lumen is unknown, however it is presumed to occur secondary to inflammation-induced mucosal damage.

IVIG for *Clostridium difficile* Infection: Outcome in Clinical Practice

In the first report by Leung et al. [25], IVIG was used for chronic relapsing CDI treatment. Its use for severe CDI was reported 7 years later [36]. Since then, a total of 15 reports have been published on IVIG for either relapsing or severe CDI treatment [25, 35–37, 47–57].

IVIG for Protracted or Relapsing CDI

The combined study population is composed of 46 patients (Table 1), described in 11 different reports: six case reports and five case series [25, 35, 37, 47, 50–55, 57]. The

average age was 63 years, (range: 1–97 years), with 58% of the patients being females. There was a large variation in IVIG dose administered with a range from 150 to 500 mg/kg over 1–6 doses. Twenty-one patients received a non-weight-based dose of 30 g for at least six doses. Treatment was successful in 40 of the 46 patients (87%), with clinical diarrhea resolution in an average of 12 days after IVIG administration (range: 1 day to 6 weeks). The patients involved were treated over an average of 168 days with different antibiotics specific for CDI (range: 3–960 days) before IVIG was given. After IVIG administration, most patients continued to receive conventional CDI treatment. All patients tested for total serum IgG or anti-toxin A IgG antibody levels (25/45 patients) had low levels. From the patients who improved with IVIG, six had recurrent diarrhea over a follow-up period of 3–24 months. Those were mostly secondary to antibiotic use for the treatment of other infections. The symptom recurrence was as early as 7 days and as late as 24 months post-treatment. Thus, the recurrence rate was 14%, which was lower compared to the recurrence rate with vancomycin and metronidazole [16].

The grade of the evidence above is weak. The available evidence is based on case reports and case series, with the largest number of patients in any study being 20. No report was controlled or randomized. Furthermore, only four papers reported both positive and negative results while seven others reported exclusively successful cases. This introduces a major bias known as the drawer effect: Not reporting or under-reporting unsuccessful cases increases the apparent effectiveness of IVIG. The same bias was introduced in all reports due to incomplete follow-up for recurrence ascertainment.

In addition, almost all patients continued to receive conventional therapy when IVIG was given. Establishing causality therefore becomes difficult, especially with the lack of controls. However, a causal relationship is likely since these same therapies failed at multiple attempts in each report to improve the disease, and because clinical cure invariably came within days of the infusion.

Although the study group was homogeneous in that CDI definition was the same in all reports (symptomatic diarrhea with positive stool toxin assay), the timing and dose of IVIG was not, and therefore generalization is difficult. No article described a clear algorithm for initiation of IVIG infusion, and the decision was left to the individual treating physician. However, the major outcome in all reports was the same (resolution of diarrhea).

Anti-Toxin A and Anti-Toxin B Monoclonal Antibodies Infusion for CDI Relapse Prevention

Lowy et al. [58] presented the results of a phase II trial in which a solution containing a combination of monoclonal antibodies against toxin A and toxin B was infused in patients suffering from CDI. It was a randomized, double-blind, placebo-controlled study performed in 30 sites in the United States and Canada. The primary endpoint was recurrence of symptomatic CDI in the 84-day follow-up period. One hundred and ninety nine patients were enrolled

Table 1 IVIG use for protracted or recurrent *C. difficile* infection: patient population

Study	Patient no.	Age	Male	Female	IVIG dose	Days to resolution[a]	Day IVIG infused[b]	Cure rate	Recurrence
Leung et al. [25][d]	5	2 (1)*	3	2	400 mg/kg every 3 weeks	42 days	120–960 days	5/5	1 of 5
Warny et al. [31][d]	1	53		1	400 mg/kg twice	7 days	150 days	1/1	0 of 1
Hassett et al. [35][d]	1	49		1	30 g every 2 weeks	n/a	720 days	1/1	1 of 1
Beales et al. [47]	4	76 (6)	1	3	400 mg/kg twice	n/a	n/a	4/4	0 of 4
Wilcox et al. [52]	5	79 (12)	1	4	300–500 mg/kg 1–6 times	1–11 days	45 to 64 days	3/5	1 of 5
Murphy et al. [51]	1	57		1	400 mg/kg 3 times	7 days	180 days	1/1	0 of 1
Cone et al. [53][d]	20	70 (10)	11	9	30 g twice	1–4 days	n/a	19/20	2 of 18
McPherson et al. [50][c]	6	81 (3)	n/a	n/a	150–400 mg/kg once	9–12 days	3–56 days	3/6	1 of 6
Chandrasekar et al. [54][c]	1	57	1		400 mg/kg 3 times	3 days	20 days	1/1	–
Groover et al. [57]	1	44		1	n/a	42 days	>21 days	1/1	n/a
Koulaouzidis et al. [55]	1	57		1	n/a	>12 days	27 days	1/1	n/a

n/a Information not available; * Standard deviation

[a] In days starting on the day IVIG was infused until the day symptoms resolved as defined in each study

[b] In days starting from the day the first CDI episode was diagnosed as defined in each study

[c] Patients in this report were divided into recurrent and fulminant CDI as defined by the authors and reported separately in Tables 1 and 2

[d] Total serum IgG or anti-toxin A IgG level was measured in the study

(99 in the study group and 100 in the control group). Traditional treatment (Vancomycin or Metronidazole) was continued in all patients. There was a significant reduction in CDI recurrence rate, with 25% recurrence in the placebo group compared to 7% in the intervention group. On the other hand, the initial episode duration, severity of symptoms, and patients' hospital stay did not differ between the intervention and the control groups. Specifically, sub-group analysis showed that patients with a single previous episode of CDI benefited from this treatment (recurrence rate of 7% compared to 38% in the control group) but not those with multiple previous episodes (7 vs. 18%, $p = 0.07$). However, the study power was only 40% to detect such a difference between the multiple recurrence vs. placebo groups. The results of this adequately designed, well-powered study, especially when validated by a phase III study, make anti-toxin A and B infusion an attractive alternative for recurrence prevention, which can be notoriously difficult to treat.

IVIG for Severe CDI

Six other reports on IVIG infusion for severe CDI were published [36, 48–50, 54, 56]: two case reports, three case series, and one case-control study. Overall, the combined study population contained 51 patients (Table 2). The average age was 68 years with 67% of the patients being females. Of note, although this percentage is higher compared to that of the relapsing CDI population, gender has not been identified as risk factor for CDI.

The definition of severe CDI varied between reports, making comparison difficult. However, the various inclusion criteria included pancolitis on CT scan either with or without megacolon [36, 48, 50], thumbprinting on CT scan [36], and a scale described by Rubin et al. [49, 59]. The most recent study, by Abougergi et al., provided two scales for inclusion: One based on extent of colonic disease and the other based on the APACHE II score to assess severity of systemic involvement [56].

There was a similar variability in IVIG dose used for severe CDI. The most frequently used dose was 400 mg/kg (range: 75–400 mg/kg) from 1–5 doses. Index hospitalization survival rate varied from 43 to 100%. The resolution of diarrhea in these cases occurred after an average of 10 days (range: 1–42 days). This is similar to the time to chronic diarrhea resolution, with a large variation depending on the patients' comorbidities. Patients received standard treatment for an average of 14 days (range: 0–65 days) before IVIG infusion. Of 51 patients, 32 survived their illness (67%). Neither total IgG nor anti-toxin A IgG levels were measured in any of the reports. Of the 32 patients who had clinical resolution, three (10%) recurred

Table 2 IVIG use for severe *C. difficile* infection: patient population

Study	Patient no.	Age	Male	Female	Severity definition	IVIG dose	Days to resolution[a]	Day IVIG infused[b]	Survival	Recurrence
Salcedo et al. [36]	2	63.5 (0.7)*	1	1	Pancolitis or thumbprinting on CAT scan	200–300 mg/kg once	1–2 days	5 and 12	2 of 2	1 out of 2
McPherson et al. [50][c]	8	72 (12)	n/a	n/a	Pancolitis	200–400 mg/kg twice	2–26 days	11–65	6 of 8	2 out of 6
Juang et al. [49]	18	67 (17.4)	5	13	Modified Rubin et al. criteria	200–300 mg/kg once	n/a	n/a	15 of 18	n/a
Hassoun et al. [48]	1	72	1	0	Pancolitis	400 mg/kg once	6 days	15	1 of 1	None
Chandrasekar et al. [54][c]	1	67	0	1	Shock requiring inotropic support and pseudomembranes on colonoscopy	400 mg/kg for 5 doses	46 days	33	1 of 1	n/a
Abougergi et al. [56]	21	68 (16)	7	14	Pancolitis and APACHE II score	300 mg/kg once—250 mg/kg for 5 doses	2–20 days	0–25	9 of 21	n/a

n/a Information not available; * Standard deviation

[a] In days starting on the day IVIG was infused until the day symptoms resolved as defined in each study

[b] In days starting from the day the first CDI episode was diagnosed as defined in each study

[c] Patients in this report were divided into recurrent and fulminant CDI as defined by the authors and reported separately in Tables 1 and 2

in a follow-up period of 1–13 months. The recurrences were at 10, 14, and 30 days post-treatment.

The same limitations discussed in the previous section also apply here. Further, generalization of the findings above is made even more difficult with the different inclusion criteria used in each report. In addition, although there was one controlled report documenting the results of IVIG use for severe CDI, propensity matching was used rather than coupled or group matching. This report was also underpowered to detect the major outcome it was designed to measure (power was 3% for an alpha of 0.05), and therefore the lack of difference between cases and controls should be interpreted with caution. Furthermore, causality is more difficult to establish in this section since most reports used much shorter conventional therapy courses before starting IVIG compared to the ones in the previous section.

Prospects for the Future

Oral Passive Immunization

Oral administration of immunoglobulin was explored in a report by Warny et al. [60]. Bovine colostrum containing anti-toxin A and anti-toxin B antibodies was administered orally to human volunteers with ileostomies. The orally administered antibodies (49%) were recovered in the ileostomy fluid, with intact toxin A-neutralizing activity. Oral immunoglobulin delivery, if effective, may provide a promising alternative to IVIG infusion due to reduced cost and increased ease of administration.

Conclusions

The efficacy of IVIG for the treatment of CDI in hamster animal models has been convincingly demonstrated. Several rigorous experiments established mechanistically and biologically that passive immunization with intravenous anti-toxin A and B antibodies is capable of both preventing and treating CDI. However, only a few small reports have documented the result of IVIG use for CDI treatment in humans to date. Those reports were mostly retrospective, non-randomized and uncontrolled. Therefore, although potentially efficacious in humans, IVIG should currently only be used as adjunct therapy until results from randomized controlled trials are available. The most evidence-based use of anti-toxin A and B antibodies to date is in purified solutions (not IVIG) for the prevention of CDI recurrence in patients who had no or one previous CDI episode.

Conflict of interest None of the authors have any conflicts of interest to declare.

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