CLOSTRIDIUM DIFFICILE ANTIBODIES

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application No. 61/526,031 filed August 22, 2011, the disclosure of which is incorporated herein by reference in its entirety.

FIELD

The invention relates to monoclonal antibodies to Clostridium difficile toxin A. The invention further relates to compositions and methods for the treatment or prevention of infection by the bacteria, Clostridium difficile, in a vertebrate subject. Methods are provided for administering antibodies to the vertebrate subject in an amount effective to reduce, eliminate, or prevent relapse from infection. Methods for the treatment or prevention of Clostridium difficile infection in an organism are provided.

BACKGROUND

Clostridium difficile (*C. difficile*) is a common nosocomial pathogen and a major cause of morbidity and mortality among hospitalized patients throughout the world. Kelly *et al.*, New Eng. J. Med., 330:257-62, 1994. The increased use of broad spectrum antibiotics and the emergence of unusually virulent strains of *C. difficile* have lead to the idea that vaccines may be well suited to reduce disease and death associated with this bacterium. *C. difficile* has few traditional antibiotic options and frequently causes a recurring disease (25% of cases). *C. difficile* claims about 20,000 lives in the USA alone per year and causes around 500,000 confirmed infections. Recently, more virulent strains of *C. difficile* have emerged that produce more toxin such as the B1/NAB1/027 strain, which also has a decreased susceptibility to metronidazole. Outbreaks of *C. difficile* have necessitated ward and partial hospital closure. With the increasing elderly population and the changing demographics of the population, *C. difficile* is set to become a major problem in the 21st century. The spectrum of *C. difficile* disease ranges from asymptomatic carriage to mild diarrhea to fulminant pseudomembranous colitis.

C. difficile has a dimorphic lifecycle whereby it exists both as an infectious and tough spore form and a metabolically active toxin-producing vegetative cell. *C. difficile*-associated disease (CDAD) is believed to be caused by the vegetative cells and more specifically the actions of two toxins, enterotoxin toxin A and cytotoxin toxin B. Vaccines and therapy for *C. difficile* have been to date focused upon the toxins (A and B), toxoids of A and B, recombinant fragments of A and B, and vegetative cell surface layer proteins (SLPAs).

Toxin A is a high-molecular weight protein that possesses multiple functional domains. The toxin is broken up into 4 functional domains: an amino-terminal glucosyltransferase that modifies Rho-like GTPases leading to cytoskeletal dysregulation in epithelial cells, an autocatalytic cysteine protease domain, a hydrophobic membrane-spanning sequence, and a highly repetitive carboxy-terminal host-cell binding domain. The carboxy terminal domain anchors the toxin to the host cell carbohydrate receptors on intestinal epithelial cells which initiates the internalization process thereby delivering the amino-terminal enzymatic domains to the cytoplasm of the target cells. The delivery of the enzymatic domain and glucosyltransferase activity leads to diarrhea and inflammation due to the apoptotic cell death of the intoxicated cells.

Many studies have shown the importance of antibodies against the toxins in affecting the disease outcome. Studies have also shown the correlation between serum anti-toxinA antibodies with protection from CDAD and relapse. These studies have led to the creation of toxin mAb therapies for CDAD.

Despite these advances, there is an unmet need for effective treatment and/or prevention of *C. difficile* associated infections including prevention from relapse of CDAD. The present invention provides mouse and humanized antibodies to toxin A to satisfy these and other needs.

SUMMARY

The present invention provides for antibodies, or antigen-binding portions thereof, that bind to *Clostridium difficile* (*C. difficile*) toxin A. The antibody or antigen-binding portion thereof may bind to fragment 4 of *C. difficile* toxin A.

In one embodiment, the present invention provides for an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain region and a light chain region, wherein the heavy chain region comprises three complementarity determining regions (CDRs), CDR1, CDR2 and CDR3, having amino acid sequences about 80% to about 100% homologous to the amino acid sequences set forth in SEQ ID NOs: 29, 30 and 31, respectively, and wherein the light chain region comprises three CDRs, CDR1, CDR2 and CDR3, having amino acid sequences about 80% to about 100% homologous to the amino acid sequences set forth in SEQ ID NOs: 21, 22 and 23, respectively.

Also provided is an isolated monoclonal antibody, or an antigen-binding portion thereof, that binds to *C. difficile* toxin A and comprises a heavy chain region, wherein the heavy chain region comprises three CDRs, CDR1, CDR2 and CDR3, having amino acid sequences about 80% to about 100% homologous to the amino acid sequences set forth in SEQ ID NOs: 29, 30 and 31, respectively.

The present invention further provides for an isolated monoclonal antibody, or an antigen-binding portion thereof, that binds to *C. difficile* toxin A and comprises a light chain region, wherein the light chain region comprises three CDRs, CDR1, CDR2 and CDR3, having amino acid sequences about 80% to about 100% homologous to the amino acid sequences set forth in SEQ ID NOs: 21, 22 and 23, respectively.

The antibody or antigen-binding portion thereof may have a dissociation constant (K_D) of less than about 1×10^{-11} M. The antibody or antigen-binding portion thereof may be humanized or chimeric.

In one embodiment, the heavy chain region of the antibody or antigen-binding portion thereof comprises an amino acid sequence about 80% to about 100% homologous to the amino acid sequence set forth in SEQ ID NO: 89; the light chain region of the antibody or antigen-binding portion thereof comprises an amino acid sequence about 80% to about 100% homologous to the amino acid sequence set forth in SEQ ID NO: 91.

In another embodiment, the heavy chain region of the antibody or antigen-binding portion thereof comprises an amino acid sequence about 80% to about 100% homologous to the amino acid sequence set forth in SEQ ID NO: 93; the light chain region of the antibody or antigen-binding portion thereof comprises an amino acid sequence about 80% to about 100% homologous to the amino acid sequence set forth in SEQ ID NO: 95.

The antibody or antigen-binding portion thereof may be the following: (a) a whole immunoglobulin molecule; (b) an scFv; (c) a Fab fragment; (d) an F(ab')2; and (e) a disulfide linked Fv.

The antibody or antigen-binding portion thereof may comprise at least one constant domain selected from the group consisting of: a) an IgG constant domain; and (b) an IgA constant domain.

One embodiment of the present invention provides for an isolated monoclonal antibody or an antigen-binding portion thereof, that binds to *C. difficile* toxin A and comprises a heavy chain variable region, wherein the heavy chain variable region comprises an amino acid sequence about 80% to about 100% homologous to the amino acid sequence set forth in SEQ ID NOs: 12, 28, 44 or 60.

Another embodiment of the present invention provides for an isolated monoclonal antibody, or an antigen-binding portion thereof, that binds to *C. difficile* toxin A and comprises a light chain variable region, wherein the light chain variable region comprises an amino acid sequence about 80% to about 100% homologous to the amino acid sequence set forth in SEQ ID NOs: 4, 20, 36 or 52.

Yet another embodiment of the present invention provides for an isolated monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody, or antigen-binding portion thereof, binds to the same epitope of *C. difficile* toxin A recognized by an antibody comprising a heavy chain variable region and a light chain variable region having amino acid sequences about 80% to about 100% homologous to the amino acid sequences set forth in SEQ ID NOs: 28 and 20, respectively.

Also encompassed by the present invention are an antibody produced by hybridoma designated CAN20G2 and the hybridoma designated CAN20G2.

The present invention provides for an isolated monoclonal antibody, or an antigen-binding portion thereof, wherein, in an *in vivo* toxin A challenge experiment,

when the antibody, or an antigen-binding portion thereof, is administered to a mammal at a dosage ranging from about 8 mg/kg body weight to about 13 mg/kg body weight about 24 hours before the mammal is exposed to greater than about 100 ng of *C. difficile* toxin A, the chance of survival for the mammal is greater than about 80% within about 7 days.

Also encompassed by the present invention is an isolated monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody, or antigen-binding portion thereof, at a concentration ranging from about 4 μM to about 17 μM, neutralizes greater than about 40% of about 150 ng/ml *C. difficile* toxin A in an *in vitro* neutralization assay.

The present invention provides for an isolated nucleic acid encoding a peptide comprising an amino acid sequence about 80% to about 100% homologous to the amino acid sequence set forth in SEQ ID NOs: 12, 28, 44, 60, 4, 20, 36 or 52. The present invention also provides for an isolated nucleic acid comprising a nucleic acid sequence about 80% to about 100% homologous to the nucleic acid sequence set forth in SEQ ID NOs: 68, 69, 70, 71, 72, 73, 74 or 75. Also provided is a cell comprising any of these nucleic acids. The cell can be a bacterial cell or a eukaryotic cell, such as a mammalian cell. Non-limiting examples of the cells include COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, SP2/0, HeLa, myeloma or lymphoma cells.

The present invention provides for a composition comprising the antibody or antigen-binding portion thereof and at least one pharmaceutically acceptable carrier.

The present invention provides for a method of preventing or treating *C. difficile*-associated disease comprising administering to a subject an effective amount of the present antibody or antigen-binding portion thereof. The antibody or antigen-binding portion thereof may be administered intravenously, subcutaneously, intramuscularly or transdermally. The method may contain another step of administering to the subject a second agent. For example, the second agent may be a different antibody or fragment thereof, or may be an antibiotic such as vancomycin, metronidazole or fidaxomicin.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a standardized ELISA showing the reactivity of purified murine mAbs on *Clostridium difficile* toxin A.

Figure 2 is an ELISA showing the binding activity of purified 1 μg/ml CAN19 mAbs on toxin A (ToxA) and toxin A fragment 4 (ToxAF4). ToxB is toxin B; ToxBF4 is toxin B fragment 4.

Figure 3 is an ELISA assay showing the binding activity of purified 1 μg/ml murine CAN20 mAbs on toxin A and toxin A fragment 4.

Figure 4 shows a Western immunoblot of Purified Murine CAN19 mAbs (0.5 μg/ml). Lane 1: Toxin A; Lane 2: Toxoid A; Lane 4: Toxin A Fragment 4; Lane 5: Toxin B; Lane 7: Toxin B Fragment 4; Lane 8: PilF (negative control). Expected sizes: Toxin A (308 kDa); Toxin A Fragment 4 (114 kDa); Toxin B (280 kDa).

Figure 5 shows a Western blot of Purified CAN20 clones (1μg/ml). Blot A was probed with CAN20G1, blot B was probed with CAN20G2, blot C was probed with CAN20G5, and blot D was probed with Can20G8. (Lane 1: Toxin A (308 kDa); Lane 2: Toxin A Fragment 4 (114 kDa); Lane3: Toxin B (280 kDa); Lane4: tetanus toxoid).

Figure 6a is an epitope binning graph showing biotinylated CAN20G1 antibody binding to SA (streptavidin) biosensor. The bound antibody is then incubated with free Toxin A and free CAN20G1. The CAN20G1-Toxin A complex is again incubated with free antibody. A large nm shift in wavelength will indicate binding of the analyte indicating that CAN20G1 and the free antibody have different epitopes. 1, Biotinylated CAN20G1 to SA biosensors. 2, Free whole toxin A forming complex with CAN20G1. 3, Free CAN20G1 associating with biotinylated CAN20G1-Toxin A complex. 4, Association sample curves. 5, Dissociation step.

Figure 6b is a graph showing the final three steps (3-5) of the full program. A large nm shift in wavelength will indicate binding of the analyte indicating that CAN20G1 and the free antibody have different epitopes. In this case, only CDA1 (Merck anti-toxin A mAb used as a control) had a significant nm shift in wavelength demonstrating that CDA1 binds to a different epitope while CAN20G1, G2, G5, and G8 bind to the same epitope bin as CAN20G1.

Figure 7 is a bar graph showing the effects of *C. difficile* toxin A on mouse survival and the efficacy of the CAN19 mAbs against the toxin A challenge.

Figure 8 is a bar graph showing the effects of *C. difficile* toxin A on mouse survival and the efficacy of the CAN19 and CAN20 mAbs against toxin A challenge.

Figure 9 is a bar graph showing the effects of *C. difficile* toxin A on mouse survival and the efficacy of the murine CAN20G2 mAb at full dose and half dose against toxin A challenge.

Figure 10 shows primers used for V gene amplification from RNA. The degenerate base symbols are IUPAC (International union of pure and applied chemistry) codes for representing degenerate nucleotide sequence patterns.

Figure 11 shows V-gene sequencing results for muCAN20G2 that includes both VH and VL sequences from the muCAN20G2 parental clones.

Figure 12 shows alignment of muCAN20G2 v-regions with the closest human germline v-region. The human germlines were used as acceptor frameworks for humanization.

Figures 13a and 13b show CDR-huCAN20G2 design. The closest matching human frameworks are IGHV7-4-1*02 and IGKV1-39*01. The CDRs (IMGT Numbering) of the muCAN20G2 were inserted into the human framework. Figure 13A shows the heavy chain variable region, including both nucleic acid sequence and amino acid sequence. FR1, FR2 and FR3 are from IGHV7-4-1*02; FR4 is from IGHJ6*01. Figure 13B shows the light (kappa) chain variable region, including both nucleic acid sequence and amino acid sequence. FR1, FR2 and FR3 are from IGKV1-39*01; FR4 is from IGKJ4*01.

Figures 14a and 14b show HE-huCAN20G2 Design. Resurfaced and altered codons are in bold. The nucleotide sequence was translated to ensure correct frame. Figure 14A shows the heavy chain variable region, including both nucleic acid sequence and amino acid sequence. Figure 14B shows the light (kappa) chain variable region, including both nucleic acid sequence and amino acid sequence.

Figure 15 shows the HE-huCAN20G2 Heavy Chain. Resurfaced and altered codons are in bold. After v-region design, an IgG1 constant region was added. The introns were removed and the nucleotide sequence was translated to ensure correct frame.

Figure 16 shows HE-huCAN20G2 Kappa Chain. Resurfaced and altered codons are in bold. After v-region design, a Kappa constant region was added. The introns were removed and the nucleotide sequence was translated to ensure correct frame.

Figure 17 shows AVA-huCAN20G2 kappa V-region alignment. The Avastin kappa v-region was aligned to the IMGT domain directory and identified the closest germline v-region. IGKV1D-33-01 was used as the acceptor framework for the AVA mAb design.

Figure 18 shows AVA-huCAN20G2. The Avastin kappa v-region was aligned to the IMGT domain directory and identified the closest germline v-region. After analysis and design, a kappa constant region was added. As previously, the constant regions contain introns. For the AVA-huCAN20G2 heavy chain, the previously designed and resurfaced HE-huCAN20G2 heavy chain was used. FR1, FR2 and FR3 are from IGKV1D-33-01; FR4 is from IGKJ1-01.

Figures 19a and 19b show chimeric CAN20G2. Murine V-regions were designed with human constant regions. The introns were removed and the nucleotide sequence was translated to ensure correct frame. Figure 19A shows the heavy chain, including both nucleic acid sequence and amino acid sequence. Figure 14B shows the light (kappa) chain, including both nucleic acid sequence and amino acid sequence.

Figure 20a shows neutralization data for purified human CAN20G2 clones at 150 ng/ml depicted as a bar graph.

Figure 20b shows neutralization data for purified human CAN20G2 clones at 250 ng/ml depicted as a bar graph.

Figure 21a shows ELISA to screen transfection supernatant for expressed human Can20G2 mAbs binding to toxin A at 45 minutes.

Figure 21b shows ELISA to screen transfection supernatant for expressed human Can20G2 mAbs binding to toxin A fragment 4 at 45 minutes.

Figure 21c shows ELISA to screen transfection supernatant for expressed human Can20G2 mAbs binding to toxin A at 60 minutes.

Figure 21d shows an ELISA to screen transfection supernatant for expressed human Can20G2 mAbs binding to toxin A fragment 4 at 60 minutes.

Figure 22 shows SDS-PAGE of purified human CAN20G2 clones.

Figure 23 shows Western blot analysis of purified human CAN20G2 clones. An SDS-page gel was run with tetanus toxoid, whole toxin A, toxin A fragment 4 and BSA. The gel was transferred to nitrocellulose membrane and probed with each of the human CAN20G2 mAbs (1μg/ml). (Lane 1: Toxin A; Lane 2: Toxin A Fragment 4; Lane 3: tetanus toxoid; Lane 4: BSA).

Figures 24a and 24b show healthy donor T cell proliferation responses to test antibodies, CDR-huCAN20G2 (Figure 24A) and HE-huCAN20G2 (Figure 24B), on days 5, 6, 7, and 8 after incubation. Proliferation responses with an SI≥2.00 (indicated by dotted line) that were significant ($p<0.05$) using an unpaired, two sample student's t test were considered positive. For each donor, the bars from left to right represent day 5, day 6, day7 and day 8, respectively.

Figure 25 shows the number of positive T cell proliferation responses to antibodies CDR-huCAN20G2 (C001) and HE-huCAN20G2 (H001) detected at four time points.

Figure 26 shows healthy donor T cell IL-2 ELISpot responses to test antibodies, CDR-huCAN20G2 (C001) and HE-huCAN20G2 (H001). PBMCs were used to assess IL-2 secretion in response to stimulation with the two antibodies during an 8-day incubation. T cell responses with an SI≥2.00 that were significant ($p<0.05$) using an unpaired, two sample student's t test were scored positive. Borderline responses (significant $p<0.05$ with SI≥1.90) was shown (*).

Figure 27 shows the comparison of HE-huCAN20G2 ("HE-CAN20G2"), CDR-huCAN20G2 ("CDR-CAN20G2") and CDA1 (Merck/Medarex) anti-*C. difficile* toxin A (anti-TcdA) mAbs tested at a low dose of 0.05mg/mouse. Efficacy of mAbs is presented as the percentages of survival compared to control animals (TcdA/PBS). *Fisher exact test for statistical significance.

Figure 28 shows the effect of humanized CAN20G2 mAbs, HE-huCAN20G2, CDR-huCAN20G2 in comparison with CDA1 on survival over time following TcdA challenge. The effect of mAbs at low dose of Ab (0.05mg) or PBS alone (control) on survival related to time after TcdA challenge is depicted. The percent survival of animals in each group post TcdA challenge at the indicated time points (hrs) is shown in the graph.

Figures 29a and 29b show PK study data of humanized antibodies CDR-huCAN20G2 (Figure 29a) and HE-huCAN20G2 (Figure 29b) in rats.

DETAILED DESCRIPTION

The present invention provides for compositions and methods for the prevention or treatment of *Clostridium difficile* bacterial infection or bacterial carriage. The compositions contain antibodies (or an antigen-binding portion thereof) that recognize toxin A of *C. difficile*, including mouse monoclonal antibodies, humanized antibodies, chimeric antibodies, or antigen-binding portions of any of the foregoing. These antibodies (or antigen-binding portion thereof) can neutralize toxin A *in vitro* and *in vivo*, and/or inhibit binding of toxin A to mammalian cells. Therefore, the present antibodies or antigen-binding portion thereof can be used in passive immunization to prevent or treat *C. difficile*-associated disease (CDAD).

In one embodiment, the present antibodies or antigen-binding portions thereof provide one or more of the following effects: protect from or treat *C. difficile*-mediated colitis, antibiotic-associated colitis, pseudomembranous colitis (PMC) or other intestinal disease in a subject; protect from or treat diarrhea in a subject; and/or treat or inhibit relapse of *C. difficile*-mediated disease. When administered to a mammal, the present antibodies or antigen-binding portions thereof protect the mammal against toxin A administered in an amount that would be fatal to the mammal had the antibody or antigen-binding portion thereof not administered.

The present antibodies or antigen-binding portions thereof include antibodies produced by hybridoma clone CAN20G2, CAN20G1, CAN20G5, CAN20G8, CAN19G1, CAN19G2 or CAN19G3 described herein.

Also encompassed by the present invention are antibodies or antigen-binding portions thereof that include an antigen-binding portion of an antibody produced by hybridoma clone CAN20G2, CAN20G1, CAN20G5, CAN20G8, CAN19G1, CAN19G2 or CAN19G3.

As used herein, CAN20G1, CAN20G2, CAN20G5, CAN20G8, CAN19G1, CAN19G2 and CAN19G3 refer to the hybridoma clones or the monoclonal antibodies generated by the corresponding hybridoma clones.

The antibodies or antigen-binding portions thereof can specifically bind to an epitope within fragment 4 of toxin A, e.g., an epitope between amino acid residues 1853-2710 of toxin A. Babcock, G.J. *et al.*, Infection and Immunity, 74: 6339-6347 (2006). In

other embodiments, the antibodies or antigen-binding portions thereof specifically bind to an epitope within fragment 1 (amino acid residues 1-659), fragment 2 (amino acid residues 660-1256) or fragment 3 (amino acid residues 1257-1852) of toxin A. In other embodiments, the antibodies or antigen-binding portions thereof specifically bind an epitope within amino acid residues 1-600, 400-600, 415-540, 1-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 900-1000, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, 1800-1900, 1900-200, 2100-2200 or 2200-2300, 2300-2400, 2400-2500, 2500-2600, 2600-2710 of toxin A, or any interval, portion or range thereof.

The present antibodies, or antigen-binding portions thereof, include, but are not limited to, monoclonal antibodies, chimeric antibodies, humanized antibodies, polyclonal antibodies, recombinant antibodies, as well as antigen-binding portions of the foregoing. An antigen-binding portion of an antibody may include a portion of an antibody that specifically binds to a toxin of *C. difficile* (e.g., toxin A).

The humanized antibody of the present invention is an antibody from a non-human species where the amino acid sequence in the non-antigen binding regions (and/or the antigen-binding regions) has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

Humanized antibodies can be generated by replacing sequences of the variable region that are not directly involved in antigen binding with equivalent sequences from human variable regions. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, may be obtained from a hybridoma producing an antibody against toxin A. The recombinant DNA encoding the humanized antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

An antibody light or heavy chain variable region consists of a framework region interrupted by three hypervariable regions, referred to as complementarity determining regions (CDRs). In one embodiment, humanized antibodies are antibody molecules from non-human species having one, two or all CDRs from the non-human species and a framework region from a human immunoglobulin molecule.

The humanized antibodies of the present invention can be produced by methods known in the art. For example, once non-human (e.g., murine) antibodies are obtained, variable regions can be sequenced, and the location of the CDRs and framework residues determined. Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242. Chothia, C. *et al.* (1987) J. Mol. Biol., 196:901-917. The light and heavy chain variable regions can, optionally, be ligated to corresponding constant regions. CDR-grafted antibody molecules can be produced by CDR-grafting or CDR substitution. One, two, or all CDRs of an immunoglobulin chain can be replaced. For example, all of the CDRs of a particular antibody may be from at least a portion of a non-human animal (e.g., mouse such as CDRs shown in Table 1) or only some of the CDRs may be replaced. It is only necessary to keep the CDRs required for binding of the antibody to a predetermined antigen (e.g., toxin A of *C. difficile*). Morrison, S. L., 1985, Science, 229:1202-1207. Oi *et al.*, 1986, BioTechniques, 4:214. U.S. Patent Nos. 5,585,089; 5,225,539; 5,693,761 and 5,693,762. EP 519596. Jones *et al.*, 1986, Nature, 321:552-525. Verhoeyan *et al.*, 1988, Science, 239:1534. Beidler *et al.*, 1988, J. Immunol., 141:4053-4060.

Also encompassed by the present invention are antibodies or antigen-binding portions thereof containing one, two, or all CDRs as disclosed herein, with the other regions replaced by sequences from at least one different species including, but not limited to, human, rabbits, sheep, dogs, cats, cows, horses, goats, pigs, monkeys, apes, gorillas, chimpanzees, ducks, geese, chickens, amphibians, reptiles and other animals.

A chimeric antibody is a molecule in which different portions are derived from different animal species. For example, an antibody may contain a variable region derived from a murine mAb and a human immunoglobulin constant region. Chimeric antibodies can be produced by recombinant DNA techniques. Morrison, et al., Proc Natl Acad Sci, 81:6851-6855 (1984). For example, a gene encoding a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted. Chimeric antibodies can also be created by recombinant DNA techniques where DNA encoding murine V regions can be ligated to DNA

encoding the human constant regions. Better et al., Science, 1988, 240:1041-1043. Liu et al. PNAS, 1987 84:3439-3443. Liu et al., J. Immunol., 1987, 139:3521-3526. Sun et al. PNAS, 1987, 84:214-218. Nishimura et al., Canc. Res., 1987, 47:999-1005. Wood et al. Nature, 1985, 314:446-449. Shaw et al., J. Natl. Cancer Inst., 1988, 80:1553-1559. International Patent Publication Nos. WO1987002671 and WO 86/01533. European Patent Application Nos. 184, 187; 171,496; 125,023; and 173,494. U.S. Patent No. 4,816,567.

The antibodies can be full-length or can include a fragment (or fragments) of the antibody having an antigen-binding portion, including, but not limited to, Fab, F(ab')2, Fab', F(ab)', Fv, single chain Fv (scFv), bivalent scFv (bi-scFv), trivalent scFv (tri-scFv), Fd, dAb fragment (e.g., Ward et al., Nature, 341:544-546 (1989)), an isolated CDR, diabodies, triabodies, tetrabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. Single chain antibodies produced by joining antibody fragments using recombinant methods, or a synthetic linker, are also encompassed by the present invention. Bird et al. Science, 1988, 242:423-426. Huston et al., Proc. Natl. Acad. Sci. USA, 1988, 85:5879-5883.

The antibodies or antigen-binding portions thereof of the present invention may be monospecific, bi-specific or multispecific. Multispecific or bi-specific antibodies or fragments thereof may be specific for different epitopes of one target polypeptide (e.g., toxin A) or may contain antigen-binding domains specific for more than one target polypeptide (e.g., antigen-binding domains specific for toxin A and toxin B; or antigen-binding domains specific for toxin A and other antigen of *C. difficile*; or antigen-binding domains specific for toxin A and other kind of bacterium or virus). In one embodiment, a multispecific antibody or antigen-binding portion thereof comprises at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Tutt et al., 1991, J. Immunol. 147:60-69. Kufer et al., 2004, Trends Biotechnol. 22:238-244. The present antibodies can be linked to or co-expressed with another functional molecule, e.g., another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or

antibody fragment to produce a bi-specific or a multispecific antibody with a second binding specificity. For example, the present invention includes bi-specific antibodies wherein one arm of an immunoglobulin is specific for toxin A, and the other arm of the immunoglobulin is specific for a second therapeutic target or is conjugated to a therapeutic moiety such as a trypsin inhibitor.

All antibody isotypes are encompassed by the present invention, including IgG (e.g., IgG1, IgG2, IgG3, IgG4), IgM, IgA (IgA1, IgA2), IgD or IgE. The antibodies or antigen-binding portions thereof may be mammalian (e.g., mouse, human) antibodies or antigen-binding portions thereof. The light chains of the antibody may be of kappa or lambda type.

The CDRs of the present antibodies or antigen-binding portions thereof can be from a non-human or human source. The framework of the present antibodies or antigen-binding portions thereof can be human, humanized, non-human (e.g., a murine framework modified to decrease antigenicity in humans), or a synthetic framework (e.g., a consensus sequence).

In one embodiment, the present antibodies, or antigen-binding portions thereof, contain at least one heavy chain variable region and/or at least one light chain variable region. The heavy chain variable region (or light chain variable region) contains three CDRs and four framework regions (FRs), arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Kabat, E. A., *et al.* Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, 1991. Chothia, C. *et al.*, J. Mol. Biol. 196:901-917, 1987.

The present antibodies or antigen-binding portions thereof specifically bind to toxin A with a dissociation constant (K_D) of less than about 10^{-7} M, less than about 10^{-8} M, less than about 10^{-9} M, less than about 10^{-10} M, less than about 10^{-11} M, or less than about 10^{-12} M.

Antibodies with a variable heavy chain region and a variable light chain region that are at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%,

about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the variable heavy chain region and variable light chain region of the antibody produced by clone CAN20G1, CAN20G2, CAN20G5, CAN20G8, CAN19G1, CAN19G2 or CAN19G3 can also bind to toxin A.

In related embodiments, anti-toxin A antibodies or antigen-binding portions thereof include, for example, the CDRs of variable heavy chains and/or variable light chains of CAN20G1, CAN20G2, CAN20G5, CAN20G8, CAN19G1, CAN19G2 or CAN19G3. The CDRs of the variable heavy chain regions from these clones, as well as the CDRs of the variable light chain regions from these clones, are shown in Table 1.

Table 1 Seq ID Nos. 3 - 104

Name	Chain, Region	Sequence	Seq ID No.
Fragment 4 of Toxin A		GWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDG VMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSK FLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNA IAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYF DTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNG FEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFD NNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDG KKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTG YTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTD ANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWR IINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQN GYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPA NTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVT GWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNL NTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKH FYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQA ILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYY FNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIIS GKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIE GQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGN RYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFK GSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIY YFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLF EIDGVIYFFGVDGVKAPGIYG	**3**
CAN20G1	K,	QVVLTQSPAIMSASLGERVTMTCTASSSVISSYLHWY	**4**

	variable region	QQKPGSSPKLWIYSTSTLASGVPARFSGSGSGTSYSLT ISSMEAEDAATYYCLQYHRSPRTFGGGTKLEIK	
CAN20G1	K, CDR1	SSVISSY	5
CAN20G1	K, CDR2	STS	6
CAN20G1	K, CDR3	CLQYHRSPRTF	7
CAN20G1	K, FR1	QVVLTQSPAIMSASLGERVTMTCTAS	8
CAN20G1	K, FR2	LHWYQQKPGSSPKLWIY	9
CAN20G1	K, FR3	TLASGVPARFSGSGSGTSYSLTISSMEAEDAATYY	10
CAN20G1	K, FR4	GGGTKLEIK	11
CAN20G1	H, variable region	QIQLVQSGPELKKPGETVKISCKASGYTFTNDGMNW VKQAPGKGLKWMGWINTNTGEPTYVEEFKGRFAFS LETSASTAYLQINNLKNEDTATYFCYVNYDYYTMDC WGQGTSVTVSS	12
CAN20G1	H, CDR1	GYTFTNDG	13
CAN20G1	H, CDR2	INTNTGEP	14
CAN20G1	H, CDR3	CYVNYDYYTMDCW	15
CAN20G1	H, FR1	QIQLVQSGPELKKPGETVKISCKAS	16
CAN20G1	H, FR2	MNWVKQAPGKGLKWMGW	17
CAN20G1	H, FR3	TYVEEFKGRFAFSLETSASTAYLQINNLKNEDTATYF	18
CAN20G1	H, FR4	GQGTSVTVSS	19
CAN20G2	K, variable region	QVVLTQSPAIMSASLGDRVTMTCTASSSVISTYLHWY QQKPGSSPKLWIYSTSTLASGVPPRFSGSGSGTSYSLT ISSMEAEDAATYYCLQYHRSPRTFGGGTKLEIK	20
CAN20G2	K, CDR1	SSVISTY	21
CAN20G2	K, CDR2	STS	22
CAN20G2	K, CDR3	LQYHRSPRT	23

CAN20G2	K, FR1	QVVLTQSPAIMSASLGDRVTMTCTAS	24
CAN20G2	K, FR2	LHWYQQKPGSSPKLWIY	25
CAN20G2	K, FR3	TLASGVPPRFSGSGSGTSYSLTISSMEAEDAATYYC	26
CAN20G2	K, FR4	FGGGTKLEIK	27
CAN20G2	H, variable region	QIQLVQSGPEVKKPGETVKISCKASGYTFTNQGMNW VKQAPGKGLKWMGWINTNTGEPTYTEEFKGRFAFSL ETSASTAYLQINNLKNEDTATYFCYVNYDYYTMDF WGQGTSVTVSS	28
CAN20G2	H, CDR1	GYTFTNQG	29
CAN20G2	H, CDR2	INTNTGEP	30
CAN20G2	H, CDR3	YVNYDYYTMDF	31
CAN20G2	H, FR1	QIQLVQSGPEVKKPGETVKISCKAS	32
CAN20G2	H, FR2	MNWVKQAPGKGLKWMGW	33
CAN20G2	H, FR3	TYTEEFKGRFAFSLETSASTAYLQINNLKNEDTATYF C	34
CAN20G2	H, FR4	WGQGTSVTVSS	35
CAN20G5	K, variable region	QIVLTQSPAIMSASLGERVTMTCTASSSVYSTYLHWY QQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSL TISSMEAEDAATYYCHQYHRSPRTFGGGTKLEIK	36
CAN20G5	K, CDR1	SSVYSTY	37
CAN20G5	K, CDR2	STS	38
CAN20G5	K, CDR3	CHQYHRSPRTF	39
CAN20G5	K, FR1	QIVLTQSPAIMSASLGERVTMTCTAS	40
CAN20G5	K, FR2	LHWYQQKPGSSPKLWIY	41
CAN20G5	K, FR3	NLASGVPARFSGSGSGTSYSLTISSMEAEDAATYY	42
CAN20G5	K, FR4	GGGTKLEIK	43

CAN20G5	H, variable region	QIQLVQSGPELKKPGETVKISCKASGYSFTNSGMNW VKEAPGKGLKWMGWINTNTGEPTYAEEFMGRFAFS LETSASTAYLQINNLKNEDTATYFCYVNYDYYTIDY WGQGTSVTVSS	44
CAN20G5	H, CDR1	GYSFTNSG	45
CAN20G5	H, CDR2	INTNTGEP	46
CAN20G5	H, CDR3	CYVNYDYYTIDYW	47
CAN20G5	H, FR1	QIQLVQSGPELKKPGETVKISCKAS	48
CAN20G5	H, FR2	MNWVKEAPGKGLKWMGW	49
CAN20G5	H, FR3	TYAEEFMGRFAFSLETSASTAYLQINNLKNEDTATYF	50
CAN20G5	H, FR4	GQGTSVTVSS	51
CAN20G8	K, variable region	QVVLTQSPAIMSASLGERVTMTCTASSSVISSYLHWY QQKPGSSPKLWIYSTSILASGVPARFSGSGSGTSYSLTI SSMEAEDAATYYCLQYHRSPRTFGGGTKLEIK	52
CAN20G8	K, CDR1	SSVISSY	53
CAN20G8	K, CDR2	STS	54
CAN20G8	K, CDR3	CLQYHRSPRTF	55
CAN20G8	K, FR1	QVVLTQSPAIMSASLGERVTMTCTAS	56
CAN20G8	K, FR2	LHWYQQKPGSSPKLWIY	57
CAN20G8	K, FR3	ILASGVPARFSGSGSGTSYSLTISSMEAEDAATYY	58
CAN20G8	K, FR4	GGGTKLEIK	59
CAN20G8	H, variable region	QIQLVQSGPELKKPGETVKISCKASGYAFTNDGMNW VKQAPGKGLKWMGWINTNTGEPTYAEEFKGRFAFS LETSASTAYLQINNLKNEDTATYFCYVNYDYYTMDC WGQGTSVTVSS	60
CAN20G8	H, CDR1	GYAFTNDG	61
CAN20G8	H, CDR2	INTNTGEP	62

CAN20G8	H, CDR3	CYVNYDYYTMDCW	63
CAN20G8	H, FR1	QIQLVQSGPELKKPGETVKISCKAS	64
CAN20G8	H, FR2	MNWVKQAPGKGLKWMGW	65
CAN20G8	H, FR3	TYAEEFKGRFAFSLETSASTAYLQINNLKNEDTATYF	66
CAN20G8	H, FR4	GQGTSVTVSS	67
CAN20G1 Kappa		Caagttgttctcacccagtctccagcaatcatgtctgcatctctaggggaacgggtca ccatgacctgcactgccagctcaagtgtaatttccagttatttgcactggtaccagcag aagccaggatcctcccccaaactctggatttatagcacatccaccctggcttctggag tcccagctcgcttcagtggcagtgggtctgggacctcttactctctcacaatcagcag catggaggctgaagatgctgccacttattactgcctccagtatcatcgttccccacgg acgttcggtggaggcaccaagctggaaatcaaacgggctgatgctgcaccaactgt atccatcttcccaccatccagtgagcagttaacatctggaggtgcctcagtcgtgtgc ttcttgaacaacttctaccccaaagacatcaatgtcaagtggaagattgatggcagtg aacgacaaaatggcgtcctgaacagttggactgatcaggacagcaaagacagcac aag	68
CAN20G1 Heavy		Cagatccagttggtgcagtctggacctgagctgaagaagcctggagagacagtca agatctcctgcaaggcttctgggtataccttcacaaacgatggaatgaactgggtga aacaggctccaggaaagggtttaaagtggatgggctggataaacaccaacactgg agagccaacatatgttgaagagttcaagggacggtttgccttctctttagaaacctctg ccagcactgcctatttgcagatcaacaacctcaaaaatgaggacacggctacatattt ctgttatgttaactacgattattatactatggactgctggggtcaaggaacctcagtcac cgtctcctcagccaaaacgacacccccatctgtctatccactggcccctggatctgct gcccaaactaactccatggtgaccctgggatgcctggtcaagggctatttccctgag ccagtgacagtgacctggaactctggatccctgtccagcggtgtgcacaccttccca gctstcctaag	69
CAN20G2 Kappa		Caagttgttctcacccagtctccagcaatcatgtctgcatctctaggggatcgggtca ccatgacctgcactgccagctcaagtgtaatttccacttacttgcactggtatcagcag aagccaggatcctcccccaaactctggatttatagcacatccaccctggcttctggag tcccacctcgcttcagtggcagtgggtctgggacctcttactctctcacaatcagcag catggaggctgaagatgctgccacttattactgcctccagtatcaccgttccccacgg acgttcggtggaggcaccaagctggaaatcaaacgggctgatgctgcaccaactgt atccatcttcccaccatccagtgagcagttaacatctggaggtgcctcagtcgtgtgc ttcttgaacaacttctaccccaaagacatcaatgtcaagtggaagattgatggcagtg aacgacaaaatggcgtcctgaacagttggactgatcaggacagcaaagacagcac aag	70
CAN20G2 Heavy		Cagatccagttggtgcagtctggacctgaggtgaagaagcctggagagacagtca agatctcctgcaaggcttctgggtataccttcacaaaccaaggaatgaactgggtga aacaggctccaggaaagggtttaaagtggatgggctggataaacaccaacactgg	71

		agagccaacatatactgaagagttcaagggacggtttgccttctctttagaaacctct gccagcactgcctatttgcagatcaacaacctcaaaaatgaggacacggctacatat ttctgttatgttaactacgattattatactatggacttctggggtcaaggaacctcggtca ccgtctcctcagccaaaacaacagccccatcggtctatccactggcccctgtgtgtg gagatacaactggctcctcggtgactctaggatgcctggtcaagggttatttccctga gccagtgaccttgacctggaactctggatccctgtccagtggtgtgcacaccttccca gctstcctaag	
CAN20G5 Kappa		Caaattgttctcacccagtctccagcaatcatgtctgcttctctaggggaacgggtca ccatgacctgcactgccagctcaagtgtatattccacttacttgcactggtaccagca gaagccaggatcctcccccaaactctggatttatagcacatccaacctggcttctgga gtcccagctcgcttcagtggcagtgggtctgggacctcttactctctcacaatcagca gcatggaggctgaagatgctgccacttattactgccaccagtatcatcgttccccacg gacgttcggtggaggcaccaagctggaaatcaaacgggctgatgctgcaccaact gtatccatcttcccaccatccagtgagcagttaacatctggaggtgcctcagtcgtgt gcttcttgaacaacttctaccccaaagacatcaatgtcaagtggaagattgatggcag tgaacgacaaaatggcgtcctgaacagttggactgatcaggacagcaaagacagc acaag	72
CAN20G5 Heavy		Cagatccagttggtacagtctggacctgagctgaagaagcctggagagacagtca agatctcctgcaaggcttctgggtattccttcacaaactctggaatgaactgggtgaa agaggctccaggaaagggtttaaagtggatgggctggataaacaccaacactgga gagccaacatatgctgaagaattcatgggacggtttgccttctctttggaaacctctgc cagcactgcctatttgcagatcaacaacctcaaaaatgaagacacggctacatatttc tgttatgttaactacgattactatactatagactactggggtcaaggaacctcagtcac cgtctcctcagccaaaacgacacccccatctgtctatccactggcccctggatctgct gcccaaactaactccatggtgaccctgggatgcctggtcaagggctatttccctgag ccagtgacagtgacctggaactctggatccctgtccagcggtgtgcacaccttccca gctstcctaag	73
CAN20G8 Kappa		Cactggtaccagcagaagccaggatcctccccaaactctggatttatagcacatc catcctggcttctggagtcccagctcgcttcagtggcagtgggtctgggacctcttac tctctcacaatcagcagcatggaggctgaagatgctgccacttattactgcctccagt atcatcgttccccacggacgttcggtggaggcaccaagctggaaatcaaacgggct gatgctgcaccaactgtatccatcttcccaccatccagtgagcagttaacatctggag gtgcctcagtcgtgtgcttcttgaacaacttctaccccaaagacatcaatgtcaagtgg aagattgatggcagtgaacgacaaaatggcgtcctgaacagttggactgatcagga cagcaaagacagcacaag	74
CAN20G8 Heavy		Cagatccagttggtgcagtctggacctgagctgaagaagcctggagagacagtca agatctcctgcaaggcttctgggtatgccttcacaaacgatggaatgaactgggtga aacaggctccaggaaagggtttaaagtggatgggctggataaacaccaacactgg agagccaacatatgctgaagagttcaagggacggtttgccttctctttagaaacctct gccagcactgcctatttgcagatcaacaacctcaaaaatgaggacacggctacatat ttctgttatgttaactacgattattatactatggactgctggggtcaaggaacctcagtc accgtctcctcagccaaaacgacacccccatctgtctatccactggcccctggatct	75

		gctgcccaaactaactccatggtgaccctgggatgcctggtcaagggctatttcct gagccagtgacagtgacctggaactctggatccctgtccagcggtgtgcacaccttc ccagctstcctaag	
5'mVK-Lead-1		GGTGCAGATTTTCAGCTTCC	**76**
3'KappaConstRT		GTGCTGTCTTTGCTGTCCTG	**77**
5'mVH-Lead-2		BTNCTYYTCTKCCTGRT	**78**
5'mVH-Lead-2A		TGGSTGTGGAMCTTGCTATT	**79**
3'mIG1-2C RT		AGGASAGCTGGGAAGGTGTG	**80**
5'mVK-Lead-3		CTWKGRSTKCTGCTKYTCTG	**81**
5'mVK-Lead-3A		CCTGTTAGGCTGTTGGTGCT	**82**
5'mVH-IGHV1-Lead		RKCARCARCTRCAGGTGTCC	**83**
5'mVH-Lead-1		CCYWNTTTTAMAWGGTGTCCAKTGT	**84**
5'mVH-Lead-3		GGATGGAGCTRTATCATBCTC	**85**
5'mVH-Lead-4		GRTCTTTMTYTTHHTCCTGTCA	**86**
5'mVH-Lead-5		VCCTTWMMTGGTATCCWGTST	**87**
CDR-huCAN20G2 (Fig.13A)	H, variable region	GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGC ACTTGTGATCTCCACCTGTCTTGAATTTTCCATGGC TCaggtgcagctggtgcaatctgggtctgagttgaagaagcctggggcctcagtg aaggtttcctgcaaggcttctGGGTATACCTTCACAAACCAAG GAAtgaattgggtgcgacaggcccctggacaagggcttgagtggatgggatgg ATAAACACCAACACTGGAGAGCCAAcgtatgcccagggctt cacaggacggtttgtcttctccttggacacctctgtcagcacggcatatctgcagatc agcagcctaaaggctgaggacactgccgtgtattactgtTATgtcaatTACGA TTATTATACTATGGACTTCtgggggcaagggaccacggtcaccgt ctcctca	**88**
CDR-huCAN20G2 (Fig.13A)	H, variable region	QVQLVQSGSELKKPGASVKVSCKASGYTFTNQGMN WVRQAPGQGLEWMGWINTNTGEPTYAQGFTGRFVF SLDTSVSTAYLQISSLKAEDTAVYYCYVNYDYYTMD FWGQGTTVTVSS	**89**
CDR-huCAN20G2	K, variable region	GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGC ACTTGTGATCTCCACCTGTCTTGAATTTTCCATGGC TGacatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagt	**90**

(Fig.13B)		caccatcacttgccgggcaagtTCAAGTGTAATTTCCACTTACT taaattggtatcagcagaaaccagggaaagcccctaagctcctgatctatAGCA CATCCAgtttgcaaagtggggtcccatcaaggttcagtggcagtggatctggg acagatttcactctcaccatcagcagtctgcaacctgaagattttgcaacttactactgt CTCCAGTATCACCGTTCCCCACGGACGttcggcggagggga ccaaggtggagatcaaa	
CDR-huCAN20G2 (Fig.13B)	K, variable region	DIQMTQSPSSLSASVGDRVTITCRASSSVISTYLNWYQ QKPGKAPKLLIYSTSSLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCLQYHRSPRTFGGGTKVEIK	**91**
HE-huCAN20G2 (Fig.14A)	H, variable region	GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGC ACTTGTGATCTCCACCTGTCTTGAATTTTCCATGGC TCAGatcCAGttgGTGcagTCTggaCCTgag**CTG**aagAAGcct GGAgagACAgtcAAGatcTCCtgcAAGgctTCTgggTATaccT TCacaAACcaaGGAatgAACtggGTGaaaCAGgctCCAggaA AGggtTTAaagTGGatgGGCtggATAaacACCaacACTggaG AGccaACAtatACT**GCCGAT**ttc**ACA**ggaCGGtttGCCttcTC TttaGAAaccTCT**GTG**AGCactGCCtatTTGcagATCaac**TC** **C**ctcAAA**GCT**GAGgacACGgctACAtatTTCtgtTATgtcaatta cGATtatTATactATGgacTTCTGGGGTCAAGGAacc**CTG** gtcACCgtcTCCtca	**92**
HE-huCAN20G2 (Fig.14A)	H, variable region	QIQLVQSGPE**L**KKPGETVKISCKASGYTFTNQGMNW VKQAPGKGLKWMGWINTNTGEPTYT**AD**F**T**GRFAFS LETS**V**STAYLQIN**S**LK**A**EDTATYFCYVNYDYYTMDF WGQGT**L**VTVSS	**93**
HE-huCAN20G2 (Fig.14B)	K, variable region	GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGC ACTTGTGATCTCCACCTGTCTTGAATTTTCCATGGC T**GAC**gtt**CAG**ctcACCcagTCTcca**AGC**atcATGtctGCAtctC TAgggGATcggGTCaccATGaccTGCactGCCagcTCAagtGT AattTCCactTACttgCACtggTATcagCAGaagCCAggaTCCtc cCCCaaaCTCtggATTtatAGCacaTCCaccCTGgctTCTggaG TCcca**AGC**cgcTTCagtGGCagtGGGtctGGGacc**GAC**tacTC TctcACAatcAGCagcATGgag**CCT**gaaGATgctGCCactTAT tacTGCctcCAGtatCACcgtTCCccaCGGacgTTCggtGGAgg cACCaag**GTG**gaaATCaaa	**94**
HE-huCAN20G2 (Fig.14B)	K, variable region	**D**V**Q**LTQSP**S**IMSASLGDRVTMTCTASSSVISTYLHWY QQKPGSSPKLWIYSTSTLASGVPPRFSGSGSGT**D**YSLT ISSME**P**EDAATYYCLQYHRSPRTFGGGTK**V**EIK	**95**
HE-huCAN20G2 (Fig.15)	H	GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGC ACTTGTGATCTCCACCTGTCTTGAATTTTCCATGGC TCAGatcCAGttgGTGcagTCTggaCCTgag**CTG**aagAAGcct GGAgagACAgtcAAGatcTCCtgcAAGgctTCTgggTATaccT TCacaAACcaaGGAatgAACtggGTGaaaCAGgctCCAggaA AGggtTTAaagTGGatgGGCtggATAaacACCaacACTggaG AGccaACAtatACT**GCCGAT**ttc**ACA**ggaCGGtttGCCttcTC	**96**

		TttaGAAaccTCTGTGAGCactGCCtatTTGcagATCaacTC CctcAAAGCTGAGgacACGgctACAtatTTCtgtTATgtcaatta cGATtatTATactATGgacTTCTGGGGTCAAGGAaccCTG gtcACCgtcTCCtcaGGTGAGTGCGGCCGCGAGCCCAG ACACTGGACGCTGAACCTCGCGGACAGTTAAGAAC CCAGGGGCCTCTGCGCCCTGGGCCCAGCTCTGTCC CACACCGCGGTCACATGGCACCACCTCTCTTGCAG CCTCCACCAAGGGCCCATCGGTCTTCCCCCTGG CACCCTCCTCCAAGAGCACCTCTGGGGGCACAG CGGCCCTGGGCTGCCTGGTCAAGGACTACTTCC CCGAACCGGTGACGGTGTCGTGGAACTCAGGC GCCCTGACCAGCGGCGTGCACACCTTCCCGGCT GTCCTACAGTCCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGC ACCCAGACCTACATCTGCAACGTGAATCACAAG CCCAGCAACACCAAGGTGGACAAGAGAGTTGGT GAGAGGCCAGCACAGGGAGGGAGGGTGTCTGCTG GAAGCCAGGCTCAGCGCTCCTGCCTGGACGCATCC CGGCTATGCAGTCCCAGTCCAGGGCAGCAAGGCAG GCCCCGTCTGCCTCTTCACCCGGAGGCCTCTGCCC GCCCCACTCATGCTCAGGGAGAGGGTCTTCTGGCT TTTTCCCCAGGCTCTGGGCAGGCACGGGCTAGGTG CCCCTAACCCAGGCCCTGCACACAAAGGGGCAGGT GCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGA GGACCCTGCCCCTGACCTAAGCCCACCCCAAAGGC CAAACTCTCCACTCCCTCAGCTCGGACACCTTCTCT CCTCCCAGATTCCAGTAACTCCCAATCTTCTCTCTG CAGAGCCCAAATCTTGTGACAAAACTCACACAT GCCCACCGTGCCCAGGTAAGCCAGCCCAGGCCTC GCCCTCCAGCTCAAGGCGGGACAGGTGCCCTAGAG TAGCCTGCATCCAGGGACAGGCCCCAGCCGGGTGC TGACACGTCCACCTCCATCTCTTCCTCAGCACCTG AACTCCTGGGGGGACCGTCAGTCTTCCTCTTCC CCCCAAAACCCAAGGACACCCTCATGATCTCCC GGACCCCTGAGGTCACATGCGTGGTGGTGGAC GTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC TGGTACGTGGACGGCGTGGAGGTGCATAATGCC AAGACAAAGCCGCGGGAGGAGCAGTACAACAG CACGTACCGTGTGGTCAGCGTCCTCACCGTCCT GCACCAGGACTGGCTGAATGGCAAGGAGTACAA GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCC CATCGAGAAAACCATCTCCAAAGCCAAAGGTGG GACCCGTGGGGTGCGAGGGCCACATGGACAGAGG CCGGCTCGGCCCACCCTCTGCCCTGAGAGTGACCG CTGTACCAACCTCTGTCCCTACAGGGCAGCCCCG AGAACCACAGGTGTACACCCTGCCCCCATCCCG	

		GGAGGAGATGACCAAGAACCAGGTCAGCCTGA CCTGCCTGGTCAAAGGCTTCTATCCCAGCGACA TCGCCGTGGAGTGGGAGAGCAATGGGCAGCCG GAGAACAACTACAAGACCACGCCTCCCGTGCTG GACTCCGACGGCTCCTTCTTCCTCTATAGCAAG CTCACCGTGGACAAGAGCAGGTGGCAGCAGGG GAACGTCTTCTCATGCTCCGTGATGCATGAGGC TCTGCACAACCACTACACGCAGAAGAGCCTCTC CCTGTCTCCGGGTAAATGATGAGCTAGC	
HE-huCAN20G2 (Fig.15)	H	QIQLVQSGPELKKPGETVKISCKASGYTFTNQGMNW VKQAPGKGLKWMGWINTNTGEPTYTADFTGRFAFS LETSVSTAYLQINSLKAEDTATYFCYVNYDYYTMDF WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVE PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK	97
HE-huCAN20G2 (Fig.16)	K	GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGC ACTTGTGATCTCCACCTGTCTTGAATTTTCCATGGC T**GAC**gtt**CAG**ctcACCcagTCTcca**AGC**atcATGtctGCAtctC TAgggGATcggGTCaccATGaccTGCactGCCagcTCAagtGT AattTCCactTACttgCACtggTATcagCAGaagCCAggcAGCt ccCCCaaaCTCtggATTtatAGCacaTCCaccCTGgctTCTgga GTCcca**AGC**cgcTTCagtGGCagtGGGtctGGGacc**GAC**tacT CTctcACAatcAGCagcATGgag**CCT**gaaGATgctGCCactTA TtacTGCctcCAGtatCACcgtTCCccaCGGacgTTCggtGGAg gcACCaag**GTG**gaaATCaaaCGTAAGTGCACTTTGCGG CCGCTAGGAAGAAACTCAAAACATCAAGATTTTAA ATACGCTTCTTGGTCTCCTTGCTATAATTATCTGGG ATAAGCATGCTGTTTTCTGTCTGTCCCTAACATGCC CTGTGATTATCCGCAAACAACACACCCAAGGGCAG AACTTTGTTACTTAAACACCATCCTGTTTGCTTCTT TCCTCAG**GAACTGTGGCTGCACCATCTGTCTTCA TCTTCCCGCCATCTGATGAGCAGTTGAAATCTG GAACTGCCTCTGTTGTGTGCCTGCTGAATAACT TCTATCCCAGAGAGGCCAAAGTACAGTGGAAGG TGGATAACGCCCTCCAATCGGGTAACTCCCAGG AGAGTGTCACAGAGCAGGACAGCAAGGACAGC ACCTACAGCCTCAGCAGCACCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCC TGCGAAGTCACCCATCAGGGCCTGAGCTCGCCC	98

		GTCACAAAGAGCTTCAACAGGGGAGAGTGTTGA TAGTTAACG	
HE-huCAN20G2 (Fig16)	K	DVQLTQSPSIMSASLGDRVTMTCTASSSVISTYLHWY QQKPGSSPKLWIYSTSTLASGVPPRFSGSGSGTDYSLT ISSMEPEDAATYYCLQYHRSPRTFGGGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC	**99**
AVA-huCAN20G2 (Fig.18)	K	GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGC ACTTGTGATCTCCACCTGTCTTGAATTTTCCATGGC TGACatcCAGatgACCcagTCTccaTCCtccCTGtctGCAtctG TAggaGACagaGTCaccATCactTGC**AGC**GCGagtTCAAG TGTAATTTCCACTTACTTAaatTGGtatCAGcagAAAcca GGGaaaGCCcctAAG**gtg**CTGatcTACAGCACATCC**AGC**t tg**cacagc**GGGgtcCCAtcaAGGttcAGTggaAGTggaTCTggg ACAgatTTTact**ctg**accATCagcAGCctgCAGcctGAAgatt**tcg** caACAtatTACtgtCTCCAGTATCACCGTTCCCCACGGA CGttcggccaagggaccaaggtggaaatcaaaCGTAAGTGCACTTT GCGGCCGCTAGGAAGAAACTCAAAACATCAAGAT TTTAAATACGCTTCTTGGTCTCCTTGCTATAATTAT CTGGGATAAGCATGCTGTTTTCTGTCTGTCCCTAAC ATGCCCTGTGATTATCCGCAAACAACACACCCAAG GGCAGAACTTTGTTACTTAAACACCATCCTGTTTGC TTCTTTCCTCAG**GAACTGTGGCTGCACCATCTGT CTTCATCTTCCCGCCATCTGATGAGCAGTTGAA ATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAA TAACTTCTATCCCAGAGAGGCCAAAGTACAGTG GAAGGTGGATAACGCCCTCCAATCGGGTAACTC CCAGGAGAGTGTCACAGAGCAGGACAGCAAGG ACAGCACCTACAGCCTCAGCAGCACCCTGACGC TGAGCAAAGCAGACTACGAGAAACACAAAGTCT ACGCCTGCGAAGTCACCCATCAGGGCCTGAGCT CGCCCGTCACAAAGAGCTTCAACAGGGGAGAGT GT**TGATAGTTAACG	**100**
Chimeric CAN20G2 (Fig.19A)	H	GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGC ACTTGTGATCTCCACCTGTCTTGAATTTTCCATGGC TCAGATCCAGTTGGTGCAGTCTGGACCTGAGGTGA AGAAGCCTGGAGAGACAGTCAAGATCTCCTGCAA GGCTTCTGGGTATACCTTCACAAACCAAGGAATGA ACTGGGTGAAACAGGCTCCAGGAAAGGGTTTAAA GTGGATGGGCTGGATAAACACCAACACTGGAGAG CCAACATATACTGAAGAGTTCAAGGGACGGTTTGC CTTCTCTTTAGAAACCTCTGCCAGCACTGCCTATTT GCAGATCAACAACCTCAAAAATGAGGACACGGCT ACATATTTCTGTTATGTTAACTACGATTATTATACT	**101**

		ATGGACTTCTGGGGTCAAGGAACCTCGGTCACCGT CTCCTCAGGTGAGTGCGGCCGCGAGCCCAGACACT GGACGCTGAACCTCGCGGACAGTTAAGAACCCAG GGGCCTCTGCGCCCTGGGCCCAGCTCTGTCCCACA CCGCGGTCACATGGCACCACCTCTCTTGCAGCCTC CACCAAGGGCCCATCGGTCTTCCCCCTGGCACC CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGC CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGA ACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT GACCAGCGGCGTGCACACCTTCCCGGCTGTCCT ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGT GGTGACCGTGCCCTCCAGCAGCTTGGGCACCCA GACCTACATCTGCAACGTGAATCACAAGCCCAG CAACACCAAGGTGGACAAGAGAGTTGGTGAGAG GCCAGCACAGGGAGGGAGGGTGTCTGCTGGAAGC CAGGCTCAGCGCTCCTGCCTGGACGCATCCCGGCT ATGCAGTCCCAGTCCAGGGCAGCAAGGCAGGCCCC GTCTGCCTCTTCACCCGGAGGCCTCTGCCCGCCCC ACTCATGCTCAGGGAGAGGGTCTTCTGGCTTTTTCC CCAGGCTCTGGGCAGGCACGGGCTAGGTGCCCCTA ACCCAGGCCCTGCACACAAAGGGGCAGGTGCTGG GCTCAGACCTGCCAAGAGCCATATCCGGGAGGACC CTGCCCCTGACCTAAGCCCACCCCAAAGGCCAAAC TCTCCACTCCCTCAGCTCGGACACCTTCTCTCCTCC CAGATTCCAGTAACTCCCAATCTTCTCTCTGCAGA GCCCAAATCTTGTGACAAAACTCACACATGCCC ACCGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCC TCCAGCTCAAGGCGGGACAGGTGCCCTAGAGTAGC CTGCATCCAGGGACAGGCCCCAGCCGGGTGCTGAC ACGTCCACCTCCATCTCTTCCTCAGCACCTGAACT CCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGAC CCCTGAGGTCACATGCGTGGTGGTGGACGTGAG CCACGAAGACCCTGAGGTCAAGTTCAACTGGTA CGTGGACGGCGTGGAGGTGCATAATGCCAAGA CAAAGCCGCGGGAGGAGCAGTACAACAGCACG TACCGTGTGGTCAGCGTCCTCACCGTCCTGCAC CAGGACTGGCTGAATGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATC GAGAAAACCATCTCCAAAGCCAAAGGTGGGACC CGTGGGGTGCGAGGGCCACATGGACAGAGGCCGG CTCGGCCCACCCTCTGCCCTGAGAGTGACCGCTGT ACCAACCTCTGTCCCTACAGGGCAGCCCCGAGAA CCACAGGTGTACACCCTGCCCCCATCCCGGGAG GAGATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCC	

		GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA CAACTACAAGACCACGCCTCCCGTGCTGGACTC CGACGGCTCCTTCTTCCTCTATAGCAAGCTCAC CGTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGC ACAACCACTACACGCAGAAGAGCCTCTCCCTGT CTCCGGGTAAATGATGA	
Chimeric CAN20G2 (Fig.19A)	H	AATMACPGFLWALVISTCLEFSMAQIQLVQSGPEVK KPGETVKISCKASGYTFTNQGMNWVKQAPGKGLKW MGWINTNTGEPTYTEEFKGRFAFSLETSASTAYLQIN NLKNEDTATYFCYVNYDYYTMDFWGQGTSVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK	**102**
Chimeric CAN20G2 (Fig.19B)	K	GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGC ACTTGTGATCTCCACCTGTCTTGAATTTTCCATGGC TCAAGTTGTTCTCACCCAGTCTCCAGCAATCATGTC TGCATCTCTAGGGGATCGGGTCACCATGACCTGCA CTGCCAGCTCAAGTGTAATTTCCACTTACTTGCACT GGTATCAGCAGAAGCCAGGcTCtTCCCCCAAACTCT GGATTTATAGCACATCCACCCTGGCTTCTGGAGTC CCACCTCGCTTCAGTGGCAGTGGGTCTGGGACCTC TTACTCTCTCACAATCAGCAGCATGGAGGCTGAAG ATGCTGCCACTTATTACTGCCTCCAGTATCACCGTT CCCCACGGACGTTCGGTGGAGGCACCAAGCTGGAA ATCAAA<u>CGTAAGTGCACTTTGCGGCCGCTAGGAAG AAACTCAAAACATCAAGATTTTAAATACGCTTCTT GGTCTCCTTGCTATAATTATCTGGGATAAGCATGCT GTTTTCTGTCTGTCCCTAACATGCCCTGTGATTATC CGCAAACAACACACCCAAGGGCAGAACTTTGTTAC TTAAACACCATCCTGTTTGCTTCTTTCCTCAG</u>**GAAC TGTGGCTGCACCATCTGTCTTCATCTTCCCGCC ATCTGATGAGCAGTTGAAATCTGGAACTGCCTC TGTTGTGTGCCTGCTGAATAACTTCTATCCCAG AGAGGCCAAAGTACAGTGGAAGGTGGATAACG CCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA CAGAGCAGGACAGCAAGGACAGCACCTACAGC CTCAGCAGCACCCTGACGCTGAGCAAAGCAGAC TACGAGAAACACAAAGTCTACGCCTGCGAAGTC	**103**

		ACCCATCAGGGCCTGAGCTCGCCCGTCACAAAG AGCTTCAACAGGGGAGAGTGTTGATAG	
Chimeric CAN20G2 (Fig.19B)	K	AATMACPGFLWALVISTCLEFSMAQVVLTQSPAIMS ASLGDRVTMTCTASSSVISTYLHWYQQKPGSSPKLWI YSTSTLASGVPPRFSGSGSGTSYSLTISSMEAEDAATY YCLQYHRSPRTFGGGTKLEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC	**104**

In Table 1, the CDRs are IMGT numbering. H: heavy chain; K: kappa chain.

In certain embodiments, the antibodies or antigen-binding portions thereof include a variable heavy chain region comprising an amino acid sequence at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to a variable heavy chain region amino acid sequence of the antibody produced by clone CAN20G1 (SEQ ID NO: 12), CAN20G2 (SEQ ID NO: 28), CAN20G5 (SEQ ID NO: 44), or CAN20G8 (SEQ ID NO: 60).

In certain embodiments, the antibodies or antigen-binding portions thereof include a variable light chain region comprising an amino acid sequence at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to a variable light chain region amino acid sequence of the antibody produced by clone CAN20G1 (SEQ ID NO: 4), CAN20G2 (SEQ ID NO: 20), CAN20G5 (SEQ ID NO: 36), or CAN20G8 (SEQ ID NO: 52).

In certain embodiments, the antibodies or antigen-binding portions thereof each include both a variable heavy chain region comprising an amino acid sequence at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%,

about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to a variable heavy chain region amino acid sequence of the antibody produced by clone CAN20G1 (SEQ ID NO: 12), CAN20G2 (SEQ ID NO: 28), CAN20G5 (SEQ ID NO: 44), or CAN20G8 (SEQ ID NO: 60), and a variable light chain region including an amino acid sequence at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to a variable light chain amino acid sequence of clone CAN20G1 (SEQ ID NO: 4), CAN20G2 (SEQ ID NO: 20), CAN20G5 (SEQ ID NO: 36), or CAN20G8 (SEQ ID NO: 52).

In various embodiments, the antibodies or antigen-binding portions thereof specifically bind to an epitope that overlaps with, or are at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to, an epitope bound by an antibody produced by clone CAN20G1, CAN20G2, CAN20G5, or CAN20G8 and/or compete for binding to toxin A with an antibody produced by clone CAN20G1, CAN20G2, CAN20G5, or CAN20G8.

A variable heavy chain region of the antibodies or antigen-binding portions thereof can comprise one, two three or more complementarity determining regions (CDRs) that are at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to CDRs of the antibody produced by clone CAN20G1 (SEQ ID NOs: 13, 14, 15),

CAN20G2 (SEQ ID NOs: 29, 30, 31), CAN20G5 (SEQ ID NOs: 45, 46, 47), or CAN20G8 (SEQ ID NOs: 61, 62, 63).

A variable light chain region of the antibodies or antigen-binding portions thereof can comprise one, two three or more CDRs that are at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to CDRs of a variable light chain region of the antibody produced by clone CAN20G1 (SEQ ID NOs: 5, 6, 7), CAN20G2 (SEQ ID NOs: 21, 22, 23), CAN20G5 (SEQ ID NOs: 37, 38, 39), or CAN20G8 (SEQ ID NOs: 53, 54, 55).

A variable heavy chain region of the antibodies or antigen-binding portions thereof can comprise one, two three or more complementarity determining regions (CDRs) that are at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to CDRs of the antibody produced by clone CAN20G1 (SEQ ID NOs: 13 - 15), CAN20G2 (SEQ ID NOs: 29 - 31), CAN20G5 (SEQ ID NOs: 45 - 47), or CAN20G8 (SEQ ID NOs: 61 - 63), and a variable light chain region of the antibodies or antigen-binding portions thereof can comprise one, two three or more CDRs that are at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to CDRs of a variable light chain region of the antibody produced by clone CAN20G1 (SEQ ID NOs: 5 - 7), CAN20G2 (SEQ ID NOs: 21 - 23), CAN20G5 (SEQ ID NOs: 37 - 39), or CAN20G8 (SEQ ID NOs: 53 - 55).

A variable heavy chain region of the antibodies or antigen-binding portions

thereof can include three CDRs that are at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to CDRs of a variable heavy chain region of the antibody produced by clone CAN20G1 (SEQ ID NOs: 13 - 15), CAN20G2 (SEQ ID NOs: 29 - 31), CAN20G5 (SEQ ID NOs: 45 - 47), or CAN20G8 (SEQ ID NOs: 61 - 63).

In one embodiment, a variable light chain region of the antibodies or antigen-binding portions thereof includes three CDRs that are at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to CDRs of a variable light chain region of the antibody produced by CAN20G1 (SEQ ID NOs: 5 - 7), CAN20G2 (SEQ ID NOs: 21 - 23), CAN20G5 (SEQ ID NOs: 37 - 39), or CAN20G8 (SEQ ID NOs: 53 - 55).

In one embodiment, a variable heavy chain region of the antibodies or antigen-binding portions thereof includes three CDRs that are at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to CDRs of a variable heavy chain region of the antibody produced by clone CAN20G1 (SEQ ID NOs: 13 - 15), CAN20G2 (SEQ ID NOs: 29 - 31), CAN20G5 (SEQ ID NOs: 45 - 47), or CAN20G8 (SEQ ID NOs: 61 - 63), and a variable light chain region of the antibodies or antigen-binding portions thereof includes one, two or three CDRs that are at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%,

about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to CDRs of a variable light chain region of the antibody produced by clone CAN20G1 (SEQ ID NOs: 5 - 7), CAN20G2 (SEQ ID NOs: 21 - 23), CAN20G5 (SEQ ID NOs: 37 - 39), or CAN20G8 (SEQ ID NOs: 53 - 55).

In certain embodiments, a variable heavy chain region of the antibodies or antigen-binding portions thereof includes three CDRs that are homologous to CDRs of a variable heavy chain region of the antibody produced by clone CAN20G1 (SEQ ID NOs: 13 - 15), CAN20G2 (SEQ ID NOs: 29 - 31), CAN20G5 (SEQ ID NOs: 45 - 47), or CAN20G8 (SEQ ID NOs: 61 - 63), and a variable light chain region of the antibodies or antigen-binding portions thereof includes three CDRs that are homologous to CDRs of a variable light chain region of the antibody produced by clone CAN20G1 (SEQ ID NOs: 5 - 7), CAN20G2 (SEQ ID NOs: 21 - 23), CAN20G5 (SEQ ID NOs: 37 - 39), or CAN20G8 (SEQ ID NOs: 53 - 55).

In certain embodiments, CDRs corresponding to the CDRs in Table 1 have sequence variations. For example, CDRs, in which 1, 2 3, 4, 5, 6, 7 or 8 residues, or less than 20%, less than 30%, or less than about 40% of total residues in the CDR, are substituted or deleted can be present in an antibody (or antigen-binding portion thereof) that binds toxin A.

In one embodiment, the antibody or antigen-binding portion thereof contains a variable light chain region and variable heavy chain region homologous to a variable light chain region and variable heavy chain region of the antibody produced by clone CAN20G1 (SEQ ID NO: 4 and SEQ ID NO:12, respectively), CAN20G2 (SEQ ID NO:20 and SEQ ID NO:28, respectively), CAN20G5 (SEQ ID NO:36 and SEQ ID NO:44, respectively), or CAN20G8 (SEQ ID NO:52 and SEQ ID NO:60, respectively).

The antibodies or antigen-binding portions thereof are peptides. The peptides may also include variants, analogs, orthologs, homologs and derivatives of peptides, that exhibit a biological activity, e.g., binding of an antigen. The peptides may contain one or more analogs of an amino acid (including, for example, non-naturally occurring amino acids, amino acids which only occur naturally in an unrelated biological system, modified amino acids from mammalian systems etc.), peptides with substituted linkages, as well as other modifications known in the art.

Also within the scope of the invention are antibodies or antigen-binding portions thereof in which specific amino acids have been substituted, deleted or added. These alternations do not have a substantial effect on the peptide's biological properties such as binding activity. For example, antibodies may have amino acid substitutions in the framework region, such as to improve binding to the antigen. In another example, a selected, small number of acceptor framework residues can be replaced by the corresponding donor amino acids. The donor framework can be a mature or germline human antibody framework sequence or a consensus sequence. Guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie *et al.*, Science, 247: 1306-1310 (1990). Cunningham *et al.*, Science, 244: 1081-1085 (1989). Ausubel (ed.), Current Protocols in Molecular Biology, John Wiley and Sons, Inc. (1994). T. Maniatis, E. F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor laboratory, Cold Spring Harbor, N.Y. (1989). Pearson, Methods Mol. Biol. 243:307-31 (1994). Gonnet *et al.*, Science 256:1443-45 (1992).

The antibody, or antigen-binding portion thereof, can be derivatized or linked to another functional molecule. For example, an antibody can be functionally linked (by chemical coupling, genetic fusion, noncovalent interaction, etc.) to one or more other molecular entities, such as another antibody, a detectable agent, a cytotoxic agent, a pharmaceutical agent, a protein or peptide that can mediate association with another molecule (such as a streptavidin core region or a polyhistidine tag), amino acid linkers, signal sequences, immunogenic carriers, or ligands useful in protein purification, such as glutathione-S-transferase, histidine tag, and staphylococcal protein A. One type of derivatized protein is produced by crosslinking two or more proteins (of the same type or of different types). Suitable crosslinkers include those that are heterobifunctional, having two distinct reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill. Useful detectable agents with which a protein can be derivatized (or labeled) include fluorescent compounds, various enzymes, prosthetic groups, luminescent materials, bioluminescent materials, and radioactive materials. Non-limiting, exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine,

and, phycoerythrin. A protein or antibody can also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, beta-galactosidase, acetylcholinesterase, glucose oxidase and the like. A protein can also be derivatized with a prosthetic group (e.g., streptavidin/biotin and avidin/biotin).

The present peptides may be the functionally active variant of antibodies of antigen-binding portions thereof disclosed herein, e.g., with less than about 30%, about 25%, about 20%, about 15%, about 10%, about 5% or about 1% amino acid residues substituted or deleted but retain essentially the same immunological properties including, but not limited to, binding to toxin A.

The invention also encompasses a nucleic acid encoding the present antibody or antigen-binding portion thereof that specifically binds to toxin A of *C. difficile*. The nucleic acid may be expressed in a cell to produce the present antibody or antigen-binding portion thereof. The isolated nucleic acid of the present invention comprises a sequence encoding a peptide at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to SEQ ID NOs: 4, 12, 20, 28, 36, 44, 52 or 60.

The isolated nucleic acid may comprise a sequence at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to SEQ ID NOs: 68, 69, 70, 71, 72, 73, 74 or 75.

The invention also features expression vectors including a nucleic acid encoding a peptide at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%,

about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to SEQ ID NOs: 4, 12, 20, 28, 36, 44, 52 or 60. The expression vector may include a nucleic acid sequence at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, about 70%, about 75%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to SEQ ID NOs: 68, 69, 70, 71, 72, 73, 74 or 75.

Nucleic acid molecules encoding a functionally active variant of the present antibody or antigen-binding portion thereof are also encompassed by the present invention. These nucleic acid molecules may hybridize with a nucleic acid encoding any of the present antibody or antigen-binding portion thereof under medium stringency, high stringency, or very high stringency conditions. Guidance for performing hybridization reactions can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. 6.3.1-6.3.6, 1989, which is incorporated herein by reference. Specific hybridization conditions referred to herein are as follows: 1) medium stringency hybridization conditions: 6XSSC at about 45°C, followed by one or more washes in 0.2XSSC, 0.1% SDS at 60°C; 2) high stringency hybridization conditions: 6XSSC at about 45°C, followed by one or more washes in 0.2XSSC, 0.1% SDS at 65°C; and 3) very high stringency hybridization conditions: 0.5 M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2XSSC, 1% SDS at 65°C.

A nucleic acid encoding the present antibody or antigen-binding portion thereof may be introduced into an expression vector that can be expressed in a suitable expression system, followed by isolation or purification of the expressed antibody or antigen-binding portion thereof. Optionally, a nucleic acid encoding the present antibody or antigen-binding portion thereof can be translated in a cell-free translation system. U.S. Patent No. 4,816,567. Queen *et al.*, Proc Natl Acad Sci USA, 86:10029-10033 (1989).

Anti-toxin antibodies or portions thereof can be produced by host cells transformed with DNA encoding light and heavy chains (or portions thereof) of a desired antibody. Antibodies can be isolated and purified from these culture supernatants and/or

cells using standard techniques. For example, a host cell may be transformed with DNA encoding the light chain, the heavy chain, or both, of an antibody. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding, e.g., the constant region.

The present nuceic acids can be expressed in various suitable cells, including prokaryotic and eukaryotic cells, e.g., bacterial cells, (e.g., E. coli), yeast cells, plant cells, insect cells, and mammalian cells. A number of mammalian cell lines are known in the art and include immortalized cell lines available from the American Type Culture Collection (ATCC). Non-limiting examples of the cells include all cell lines of mammalian origin or mammalian-like characteristics, including but not limited to, parental cells, derivatives and/or engineered variants of monkey kidney cells (COS, e.g., COS-1, COS-7), HEK293, baby hamster kidney (BHK, e.g., BHK21), Chinese hamster ovary (CHO), NS0, PerC6, BSC-1, human hepatocellular carcinoma cells (e.g., Hep G2), SP2/0, HeLa, Madin-Darby bovine kidney (MDBK), myeloma and lymphoma cells. The engineered variants include, e.g., glycan profile modified and/or site-specific integration site derivatives.

The present invention also provides for cells comprising the nucleic acids described herein. The cells may be a hybridoma or transfectant. The types of the cells are discussed above.

The present antibody or antigen-binding portion thereof can be expressed in various cells. The types of the cells are discussed above.

Alternatively, the present antibody or antigen-binding portion thereof can be synthesized by solid phase procedures well known in the art. Solid Phase Peptide Synthesis: A Practical Approach by E. Atherton and R. C. Sheppard, published by IRL at Oxford University Press (1989). Methods in Molecular Biology, Vol. 35: Peptide Synthesis Protocols (ed. M. W.Pennington and B. M. Dunn), chapter 7. Solid Phase Peptide Synthesis, 2nd Ed., Pierce Chemical Co., Rockford, IL (1984). G. Barany and R. B. Merrifield, The Peptides: Analysis, Synthesis, Biology, editors E. Gross and J. Meienhofer, Vol. 1 and Vol. 2, Academic Press, New York, (1980), pp. 3-254. M. Bodansky, Principles of Peptide Synthesis, Springer-Verlag, Berlin (1984).

The present invention provides for methods for making an antibody or antigen-binding portion thereof that specifically binds to toxin A of *C. difficile*. For example, a non-human animal is immunized with a composition that includes an inactivated toxin A, and then a specific antibody is isolated from the animal. The method can further include evaluating binding of the antibody to toxin A.

Any of a variety of *Clostridium difficile* toxin proteins, particularly toxin A, may be used in the practice of the present invention. *C. difficile* disease is mediated primarily by toxin A and toxin B. Both toxins are cytotoxic, and lethal when injected intravenously or intraperitoneally into a mouse. Toxin A is also a potent enterotoxin, as demonstrated by the induction of fluid accumulation in the mouse ligated intestinal loop diarrhea model. See, e.g., Babcock, G.J. *et al.*, Infection and Immunity, 74: 6339-6347 (2006) and references contained therein for background.

Table 2 provides amino acid sequences of *Clostridium difficile* toxin A. Variants and fragments of the sequences provided below can also be used as an antigen to generate antibodies.

Table 2

SEQ ID NO	Protein Name	Amino acid Sequence
1	Toxin A	MSLISKEELIKLAYSIRPRENEYKTILTNLDEYNKLTTNNNEN KYLQLKKLNESIDVFMNKYKNSSRNRALSNLKKDILKEVILI KNSNTSPVEKNLHFVWIGGEVSDIALEYIKQWADINAEYNIK LWYDSEAFLVNTLKKAIVESSTTEALQLLEEEIQNPQFDNMK FYKKRMEFIYDRQKRFINYYKSQINKPTVPTIDDIIKSHLVSEY NRDETLLESYRTNSLRKINSNHGIDIRANSLFTEQELLNIYSQE LLNRGNLAAASDIVRLLALKNFGGVYLDVDMLPGIHSDLFK TIPRPSSIGLDRWEMIKLEAIMKYKKYINNYTSENFDKLDQQL KDNFKLIIESKSEKSEIFSKLENLNVSDLEIKIAFALGSVINQAL ISKQGSYLTNLVIEQVKNRYQFLNQHLNPAIESDNNFTDTTKI FHDSLFNSATAENSMFLTKIAPYLQVGFMPEARSTISLSGPGA YASAYYDFINLQENTIEKTLKASDLIEFKFPENNLSQLTEQEIN SLWSFDQASAKYQFEKYVRDYTGGSLSEDNGVDFNKNTAL DKNYLLNNKIPSNNVEEAGSKNYVHYIIQLQGDDISYEATCN LFSKNPKNSIIIQRNMNESAKSYFLSDDGESILELNKYRIPERL KNKEKVKVTFIGHGKDEFNTSEFARLSVDSLSNEISSFLDTIK LDISPKNVEVNLLGCNMFSYDFNVEETYPGKLLLSIMDKITST LPDVNKDSITIGANQYEVRINSEGRKELLAHSGKWINKEEAI MSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDAS

SEQ ID NO	Protein Name	Amino acid Sequence
		VSPDTKFILNNLKLNIESSIGDYIYYEKLEPVKNIIHNSIDDLID EFNLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRF INKSNGESVYVETEKEIFSKYSEHITKEISTIKNSIITDVNGNLL DNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTSVKV QLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIV STILDGINLGAAIKELLDEHDPLLKKELEAKVGVLAINMSLSI AATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSV VNYFNHLSESKEYGPLKTEDDKILVPIDDLVISEIDFNNNSIKL GTCNILAMEGGSGHTVTGNIDHFFSSPYISSHIPSLSVYSAIGI KTENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENNGTK LLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNTKIKLD KDTRNFIMPTITTDEIRNKLSYSFDGAGGTYSLLLSSYPISMNI NLSKDDLWIFNIDNEVREISIENGTIKKGNLIEDVLSKIDINKN KLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSY SLLLSGDKNYLISNLSNTIEKINTLGLDSKNIAYNYTDESNNK YFGAISKTSQKSIIHYKKDSKNILEFYNGSTLEFNSKDFIAEDI NVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGL YLNESVYSSYLDFVKNSDGHHNTSNFMNLFLNNISFWKLFGF ENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSS SKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRY INKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNI NLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNT QSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKI IDNKTYYYDEDSKLVKGLININNSLFYFDPIESNLVTGWQTIN GKKYYFDINTGAASTSYKIINGKHFYFNNNGVMQLGVFKGP DGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDS KAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDT AIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDC VVKIGVFSGSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNG KKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTID GKKYYFNTNTAEAATGWQTIDGKKYYFNTNTSIASTGYTIIN GKYFYFNTDGIMQIGVFKVPNGFEYFAPANTHNNNIEGQAIL YQNKFLTLNGKKYYFGSDSKAITGWQTIDGKKYYFNPNNAI AATHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESK MVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNG KKYYFDNDSKAVTGWQTIDSKKYYFNLNTAVAVTGWQTID GEKYYFNLNTAEAATGWQTIDGKRYYFNTNTYIASTGYTIIN GKHFYFNTDGIMQIGVFKGPDGFEYFAPANTHNNNIEGQAIL YQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAV AVTGWQTINGKKYYFNTNTYIASTGYTIISGKHFYFNTDGIM QIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNI YYFGNDSKAATGWATIDGNRYYFEPNTAMGANGYKTIDNK NFYFRNGLPQIGVFKGPNGFEYFAPANTDANNIDGQAIRYQN

SEQ ID NO	Protein Name	Amino acid Sequence
		RFLHLLGKIYYFGNNSKAVTGWQTINSKVYYFMPDTAMAA AGGLFEIDGVIYFFGVDGVKAPGIYG

Table 3 provides nucleic acid sequences encoding the proteins of Table 2.

Table 3

SEQ ID NO	Accession Number And Gene Name	Nucleotide Sequence
2	Toxin A	atgtctttaa tatctaaaga agagttaata aaactcgcat atagcattag accaagagaa aatgagtata aaactatact aactaattta gacgaatata ataagttaac tacaaacaat aatgaaaata aatatttaca attaaaaaaa ctaaatgaat caattgatgt ttttatgaat aaatataaaa attcaagcag aaatagagca ctctctaatc taaaaaaaga tatattaaaa gaagtaattc ttattaaaaa ttccaataca agtcctgtag aaaaaaattt acattttgta tggataggtg gagaagtcag tgatattgct cttgaataca taaaacaatg ggctgatatt aatgcagaat ataatattaa actgtggtat gatagtgaag cattcttagt caatacacta aaaaaggcta tagttgaatc ttctaccact gaagcattac agctactaga ggaagagatt caaaatcctc aatttgataa tatgaaattt tacaaaaaaa ggatggaatt tatatatgat agacaaaaaa ggtttataaa ttattataaa tctcaaatca ataaacctac agtacctaca atagatgata ttataaagtc tcatctagta tctgaatata atagagatga aactttatta gaatcatata gaacaaattc tttgagaaaa ataaatagta atcatgggat agatatcagg gctaatagtt tgtttacaga acaagagtta ttaaatattt atagtcagga gttgttaaat cgtgggaatt tagctgcagc atctgacata gtaagattat tagccctaaa aaattttggc ggagtatatt tagatgttga tatgcttcca ggtattcact ctgatttatt taaaacaata cctagaccta gctctattgg actagaccgt tgggaaatga taaaattaga ggctattatg aagtataaaa aatatataaa taattataca tcagaaaact ttgataaact tgatcaacaa ttaaaagata attttaaact cattatagaa agtaaaagtg aaaaatctga gatattttct aaattagaaa atttaaatgt atctgatctt gaaattaaaa tagctttcgc tttaggcagt gttataaatc aagccttgat atcaaaacaa ggttcatatc ttactaacct agtaatagaa caagtaaaaa atagatatca atttttaaac caacacctta acccagccat agagtctgac aataacttca cagatactac taagattttt catgattcac tatttaattc agctaccgca gaaaactcta tgtttttaac aaaaatagca ccatacttac aagtaggttt tatgccagaa gctcgctcca caataagttt aagtggtcca ggagcttatg catcagctta ctatgatttc ataaatttac aagaaaatac tatagaaaaa actttaaaag catcagattt aatagaattt aaattcccag aaaataatct atctcaattg acagaacaag aaataaatag tctatggagc tttgatcaag caagtgcaaa atatcaattt gagaaatatg taagagatta tactggtgga tctctttctg aagacaatgg ggtagacttt aataaaaata ctgccctcga caaaaactat ttattaaata ataaaattcc atcaaacaat gtagaagaag ctggaagtaa aaattatgtt cattatatca tacagttaca aggagatgat ataagttatg aagcaacatg caatttattt tctaaaaatc ctaaaaatag tattattata caacgaaata tgaatgaaag tgcaaaaagt tacttttaa gtgatgatgg agaatctatt ttagaattaa ataaatatag gatacctgaa agattaaaaa ataaggaaaa agtaaaagta acctttattg gacatggtaa agatgaattc aacacaagcg aatttgctag attaagtgta gattcacttt ccaatgagat aagttcattt

Table 3		
SEQ ID NO	**Accession Number And Gene Name**	**Nucleotide Sequence**
		ttagatacca taaaattaga tatatcacct aaaaatgtag aagtaaactt gcttggatgt aatatgttta gttatgattt taatgttgaa gaaacttatc ctggtaagtt actattaagt attatggaca aaattacttc cactttacct gatgtaaata aagattctat tactatagga gcaaatcaat atgaagtaag aattaatagt gagggaagaa aagaacttct agctcactca ggtaaatgga taaataaaga ggaagctatt atgagcgatt tatctagtaa agaatacatt ttttttgatt ccatagataa taagctaaaa gcaaagtcca agaatattcc aggtttagcg tcaatatcag aagatataaa aacattatta cttgatgcaa gtgttagtcc tgatacaaaa tttattttaa ataatcttaa gcttaatatt gaatcttcta ttggtgatta catttattat gaaaaattag aacctgttaa aaatataatc cacaattcta tagatgattt aatagatgag ttcaatctac ttgaaaatgt atctgatgaa ttatatgaat taaaaaaatt aaataatcta gatgagaagt atttaatatc ttttgaagat atctcaaaaa ataattcaac ttattctgta agatttatta acaaaagtaa tggtgaatca gtttatgtag agacagaaaa agaaattttt tcaaaatata gcgaacatat tacaaaagaa ataagtacta taaagaatag tataattaca gatgttaatg gtaatttatt ggataatata cagttagatc atacttctca agttaataca ttaaacgcag cattctttat tcaatcatta atagattata gtagcaataa agatgtactg aatgatttaa gtacctcagt taaggttcaa ctttatgctc aactatttag tacaggttta aatactatat atgactctat ccaattagta aatttaatat caaatgcagt aaatgatact ataaatgtac tacctacaat aacagagggg atacctattg tatctactat attagacgga ataaacttag gtgcagcaat taaggaatta ctagacgaac atgacccatt actaaaaaaa gaactagaag ctaaggtggg tgttttagca ataaatatgt cattatctat agctgcaacg gtagcttcaa ttgttggaat aggtgctgaa gttactattt tcttattacc tatagctggt atatctgcgg gaataccttc attagttaat aatgaattaa tattgcatga taaggcaact tcagtggtaa actattttaa tcatttgtct gaatctaaag aatatggccc tcttaaaaca gaagatgata aaattttagt tcctattgat gatttagtaa tatcagaaat agattttaat aataattcga taaaactagg aacatgtaat atattagcaa tggaggggggg atcaggacac acagtgactg gtaatataga tcacttttc tcatctccat atataagctc tcatattcct tcattatcag tttattctgc aataggtata aaaacagaaa atctagattt ttcaaaaaaa ataatgatgt taccaaatgc tccttcaaga gtgttttggt gggaaactgg agcagttcca ggtttaagat cattggaaaa taatgggact aaattgcttg attcaataag agatttatac ccaggcaaat tttactggag attctatgcc tttttcgatt atgcaataac tacattaaaa ccagtgtatg aagacactaa tactaaaatt aaactagata aagatactag aaactttata atgccaacta taactactga cgaaattaga aacaaattat cttattcatt tgatggagca ggaggaactt actctttatt attatcttca tatccaatat caatgaatat aaatttatct aaagatgatt tatggatatt taatattgat aatgaagtaa gagaaatatc tatagaaaat ggtactatta aaaaaggaaa tttaatagaa gatgttttaa gtaaaattga tataaataaa aataaactta ttataggcaa tcaaacaata gatttttcag gtgatataga taacaaagat agatatatat tcttgacttg tgagttagat gataaaatta gtttaataat agaaataaat cttgttgcaa aatcttatag tttgttattg tctggggata aaaattattt gatatccaat ttatctaata ctattgagaa aatcaatact ttaggcctag atagtaaaaa tatagcttac aattacactg atgaatctaa taataaatat tttggagcta tatctaaaac aagtcaaaaa agcataatac attataaaaa agacagtaaa aatatattag aattttataa tggcagtaca

Table 3		
SEQ ID NO	**Accession Number And Gene Name**	**Nucleotide Sequence**
		ttagaattta acagtaaaga ctttattgct gaagatataa atgtatttat gaaagatgat attaatacta taacaggaaa atactatgtt gataataata ctgataaaag tatagatttc tctatttctt tagttagtaa aaatcaagta aaagtaaatg gattatattt aaatgaatcc gtatactcat cttaccttga ttttgtgaaa aattcagatg gacaccataa tacttctaat tttatgaatt tatttttgaa caatataagt ttctggaaat tgtttgggtt tgaaaatata aattttgtaa tcgataaata ctttaccctt gttggtaaaa ctaatcttgg atatgtagaa tttatttgtg acaataataa aaatatagat atatattttg gtgaatggaa aacatcgtca tctaaaagca ctatatttag cggaaatggt agaaatgttg tagtagagcc tatatataat cctgatacgg gtgaagatat atctacttca ctagattttt cctatgaacc tctctatgga atagatagat atatcaataa agtattgata gcacctgatt tatatacaag tttaataaat attaatacca attattattc aaatgagtac taccctgaga ttatagttct taacccaaat acattccaca aaaaagtaaa tataaattta gatagttctt cttttgagta taaatggtct acagaaggaa gtgactttat tttagttaga tacttagaag aaagtaataa aaaaatatta caaaaaataa gaatcaaagg tatcttatct aatactcaat catttaataa aatgagtata gattttaaag atattaaaaa actatcatta ggatatataa tgagtaattt taaatcattt aattctgaaa atgaattaga tagagatcat ttaggattta aaataataga taataaaact tattactatg atgaagatag taaattagtt aaaggattaa tcaatataaa taattcatta ttctattttg atcctataga atctaactta gtaactggat ggcaaactat caatggtaaa aaatattatt ttgatataaa tactggagca gcttcaacta gttataaaat tattaatggt aaacactttt attttaataa taatggtgtg atgcagttag gagtatttaa aggacctgat ggatttgagt attttgcacc tgccaatact cagaataata acatagaagg tcaggctata gtttatcaaa gtaaattctt aactttgaat ggcaaaaaat attattttga taatgactca aaagcagtca ctggatggag gattattaac aatgagaaat attactttaa tcctaataat gctattgctg cagtcggatt gcaagtaatt gacaataata agtattattt caatcctgac actgctatca tctcaaaagg ttggcagact gttaatggta gtagatacta ctttgatact gataccgcta ttgcctttaa tggttataaa actattgatg gtaaacactt ttattttgat agtgattgtg tagtgaaaat aggtgtgttt agtggctcta atggatttga atatttcgca cctgctaata cttataataa taacatagaa ggtcaggcta tagtttatca aagtaaattc ttaactttga atggtaaaaa atattacttt gataataact caaaagcagt taccggatgg caaactattg atagtaaaaa atattacttt aatactaaca ctgctgaagc agctactgga tggcaaacta ttgatggtaa aaagtattac tttaatacta acactgctga agcagctact ggatggcaaa ctattgatgg taaaaaatat tactttaata ctaacacttc tatagcttca actggttata caattattaa tggtaaatat ttttatttta atactgatgg tattatgcag ataggagtgt ttaaagtacc taatggattt gaatactttg cacctgctaa tactcataat aataacatag aaggtcaagc tatactttac caaaataaat tcttaacttt gaatggtaaa aaatattact ttggtagtga ctcaaaagca attactggat ggcaaaccat tgatggtaaa aaatattact ttaatcctaa taatgctatt gctgcgactc atctatgcac tataaataac gacaagtatt actttagtta tgatggaatt cttcaaaatg gatatattac tattgaaaga aataatttct attttgatgc taataatgaa tctaaaatgg taacaggagt atttaaagga cctaatggat ttgagtattt tgcacctgct aatactcata ataataacat agaaggtcag gctatagttt accagaataa attcttaact ttgaatggca aaaaatatta ttttgataat

Table 3		
SEQ ID NO	**Accession Number And Gene Name**	**Nucleotide Sequence**
		gactcaaaag cagttactgg atggcaaact attgatagta aaaaatatta ctttaatctt aacactgctg ttgcagttac tggatggcaa actattgatg gtgaaaaata ttactttaat cttaacactg ctgaagcagc tactggatgg caaactattg atggtaaaag atactacttt aatactaaca cttatatagc ttcaactggt tatacgatta ttaatggtaa acatttttat tttaatactg atggtattat gcagatagga gtgtttaaag gacctgatgg atttgaatac tttgcacctg ctaatactca taataataac atagaaggtc aagctatact ttaccaaaat aaattcttaa ctttgaatgg taaaaaatat tactttggta gtgactcaaa agcagttacc ggattgcgaa ctattgatgg taaaaaatat tactttaata ctaacactgc tgttgcagtt actggatggc aaactattaa tggtaaaaaa tactacttta atactaacac ttatatagct tcaactggtt atacaattat tagtggtaaa catttttatt ttaatactga tggtattatg cagataggag tgtttaaagg acctgatgga tttgaatact ttgcacctgc taatacggat gctaacaaca tagaaggtca agctatacgt tatcaaaata gattcctata tttacatgac aatatatatt actttggcaa tgattcaaaa gcggctactg gttgggcaac tattgatggt aatagatatt acttcgagcc taatacagct atgggtgcga atggttataa aactattgat aataaaaatt tttactttag aaatggttta cctcagatag gagtgtttaa aggacctaat ggatttgaat actttgcacc tgctaatacg gatgctaaca atatagatgg tcaagctata cgttatcaaa atagattcct acatttactt ggaaaaatat attactttgg taataactca aaagcagtta ctggatggca aactattaat agtaaagtat attactttat gcctgatact gctatggctg cagctggtgg actttcgag attgatggtg ttatatattt ctttggtgtt gatggagtaa aagcccctgg gatatatggc taa

In one embodiment, the present invention provides for a method for making a hybridoma that expresses an antibody that specifically binds to toxin A of *C. difficile*. The method contains the following steps: immunizing an animal with a composition that includes inactivated toxin A (e.g., toxoid A); isolating splenocytes from the animal; generating hybridomas from the splenocytes; and selecting a hybridoma that produces an antibody that specifically binds to toxin A. Kohler and Milstein, Nature, 256: 495, 1975. Harlow, E. and Lane, D. Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988.

Toxins can be inactivated, for example, by treatment with formaldehyde, glutaraldehyde, UDP-dialdehyde, peroxide, oxygen or by mutation (e.g., using recombinant methods). Relyveld *et al.*, Methods in Enzymology, 93:24, 1983. Woodrow and Levine, eds., New Generation Vaccines, Marcel Dekker, Inc., New York, 1990. Genth *et al.*, Inf. and Immun., 68(3):1094-1101, 2000. Mutant *C. difficile* toxins with

reduced toxicity can be produced using recombinant methods. U.S. Patent Nos. 5,085,862; 5,221,618; 5,244,657; 5,332,583; 5,358,868; and 5,433,945. A full-length or fragment of the toxins or toxoids can be used as immunogens.

In one embodiment, inactivated toxin A is used to immunize mice intraperitoneally or intravenously. One or more boosts may or may not be given. The titers of the antibodies in the plasma can be monitored by, e.g., ELISA (enzyme-linked immunosorbent assay) or flow cytometry. Mice with sufficient titers of anti-toxin A antibodies are used for fusions. Mice may or may not be boosted with antigen 3 days before sacrifice and removal of the spleen. The mouse splenocytes are isolated and fused with PEG to a mouse myeloma cell line. The resulting hybridomas are then screened for the production of antigen-specific antibodies. Cells are plated, and then incubated in selective medium. Supernatants from individual wells are then screened by ELISA for human anti-toxin monoclonal antibodies. The antibody secreting hybridomas are replated, screened again, and if still positive for anti-toxin monoclonal antibodies, can be subcloned by limiting dilution. For example, the hybridoma clone CAN20G2 of the present invention has been subcloned. One of the subclones is CAN20G2-2-1.

Adjuvants that may be used to increase the immunogenicity of one or more of the *Clostridium difficile* toxin antigens, particularly toxin A include any compound or compounds that act to increase an immune response to peptides or combination of peptides. Non-limiting examples of adjuvants include alum, aluminum phosphate, aluminum hydroxide, MF59 (4.3% w/v squalene, 0.5% w/v polysorbate 80 (Tween 80), 0.5% w/v sorbitan trioleate (Span 85)), CpG-containing nucleic acid, QS21 (saponin adjuvant), MPL (Monophosphoryl Lipid A), 3DMPL (3-O-deacylated MPL), extracts from Aquilla, ISCOMS (see, e.g., Sjolander *et al.* (1998) J. Leukocyte Biol. 64:713; WO90/03184; WO96/11711; WO 00/48630; WO98/36772; WO00/41720; WO06/134423 and WO07/026190), LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, Quil A, interleukins, Freund's, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dip- almitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria,

monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion.

The immunized animal can be any animal that is capable of producing recoverable antibodies when administered an immunogen, such as, but not limited to, rabbits, mice, rats, hamsters, goats, horses, monkeys, baboons and humans. In one aspect, the host is transgenic and produces human antibodies, e.g., a mouse expressing the human immunoglobulin gene segments. U.S. Patent No. 8,236,311; 7,625,559 and 5,770,429, the disclosure of each of which is incorporated herein by reference in its entirety. Lonberg *et al.*, Nature 368(6474): 856-859, 1994. Lonberg, N., Handbook of Experimental Pharmacology 113:49-101, 1994. Lonberg, N. and Huszar, D., Intern. Rev. Immunol., 13: 65-93, 1995. Harding, F. and Lonberg, N., Ann. N.Y. Acad. Sci., 764:536-546, 1995.

After the host is immunized and the antibodies are produced, the antibodies are assayed to confirm that they are specific for the antigen of interest and to determine whether they exhibit any cross reactivity with other antigens. One method of conducting such assays is a sera screen assay as described in U.S. Patent Publication No. 2004/0126829. Anti-toxin antibodies can be characterized for binding to the toxin by a variety of known techniques. For example, in an ELISA, microtiter plates are coated with the toxin or toxoid antigen in PBS, and then blocked with irrelevant proteins such as bovine serum albumin (BSA) diluted in PBS. Dilutions of plasma from toxin-immunized mice are added to each well and incubated. The plates are washed and then incubated with a secondary antibody conjugated to an enzyme (e.g., alkaline phosphatase). After washing, the plates are developed with the enzyme's substrate (e.g., ABTS), and analyzed at a specific OD. In other embodiments, to determine if the selected monoclonal antibodies bind to unique epitopes, the antibody can be biotinylated which can then be detected with a streptavidin labeled probe. Anti-toxin antibodies can be tested for reactivity with the toxin by Western blotting.

Neutralization assays can also be used to measure activity of the anti-toxin antibodies. For example, *in vitro* neutralization assays can be used to measure the ability of an antibody to inhibit a cytopathic effect on cells in culture (see Examples 7 and 12

below). In one embodiment, the present antibody, or antigen-binding portion thereof, at a concentration ranging from about 1 μM to about 50 μM, from about 2 μM to about 40 μM, from about 3 μM to about 30 μM, from about 4 μM to about 20 μM, from about 4 μM to about 17 μM, from about 5 μM to about 15 μM, or about 10 μM neutralizes greater than about 20%, greater than about 30%, greater than about 40%, greater than about 50%, greater than about 60%, greater than about 70%, greater than about 80%, or greater than about 90% of about 150 ng/ml *C. difficile* toxin A in an *in vitro* neutralization assay. In vivo assays can be used to measure toxin neutralization as well. In another embodiment, in an *in vivo* toxin A challenge experiment (e.g., procedures as described in Examples 5, 6, and 7, as well as Babcock *et al.*, Human Monoclonal Antibodies Directed against Toxins A and B prevent *Clostridium difficile*-Induced Mortality in Hamsters. Infection and Immunity (2006) 74(11):6339), when the antibody, or an antigen-binding portion thereof, is administered to a mammal at a dosage ranging from about 1 mg/kg body weight to about 50 mg/kg body weight, from about 2 mg/kg body weight to about 40 mg/kg body weight, from about 3 mg/kg body weight to about 30 mg/kg body weight, from about 5 mg/kg body weight to about 20 mg/kg body weight, from about 8 mg/kg body weight to about 13 mg/kg body weight, or about 10 mg/kg body weight about 24 hours before the mammal is exposed to greater than about 100 ng, or about 100 ng of *C. difficile* toxin A, the chance of survival for the mammal is greater than about 40%, greater than about 50%, greater than about 60%, greater than about 70%, greater than about 80%, or greater than about 90% within about 7 days.

Hybridomas that produce antibodies that bind, preferably with high affinity, to the toxin can than be subcloned and further characterized. One clone from each hybridoma, which retains the reactivity of the parent cells (by ELISA), can then be chosen for making a cell bank, and for antibody purification.

To purify the anti-toxin antibodies, supernatants from the cultured hybridomas can be filtered and concentrated before affinity chromatography with protein A-Sepharose (Pharmacia, Piscataway, N.J.).

Antibodies, or antigen-binding fragments, variants or derivatives thereof of the present disclosure can also be described or specified in terms of their binding affinity to

an antigen. The affinity of an antibody for an antigen can be determined experimentally using any suitable method (see, *e.g.,* Berzofsky *et al.*, "Antibody-Antigen Interactions," In Fundamental Immunology, Paul, W. E., Ed., Raven Press: New York, N.Y. (1984); Kuby, Janis Immunology, W. H. Freeman and Company: New York, N.Y. (1992); and methods described herein). The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions (*e.g.,* salt concentration, pH). Thus, measurements of affinity and other antigen-binding parameters (*e.g.,* K_D, K_a, K_d) are preferably made with standardized solutions of antibody and antigen, and a standardized buffer.

The present antibodies or antigen-binding portions thereof have *in vitro* and *in vivo* therapeutic, prophylactic, and/or diagnostic utilities. For example, these antibodies can be administered to cells in culture, e.g., *in vitro* or ex vivo, or to a subject, e.g., *in vivo*, to treat, inhibit, prevent relapse, and/or diagnose *C. difficile* and disease associated with *C. difficile*.

The antibodies or antigen-binding portions thereof can be used on cells in culture, e.g., *in vitro* or ex vivo. For example, cells can be cultured *in vitro* in culture medium and contacted by the anti-toxin antibody or fragment thereof. The methods can be performed on cells present in a subject, as part of an *in vivo* (e.g., therapeutic or prophylactic) protocol. For *in vivo* embodiments, the contacting step is effected in a subject and includes administering an anti-toxin antibody or portion thereof to the subject under conditions effective to permit binding of the antibody, or portion thereof, to a toxin (e.g., toxin A) expressed by *C. difficile* in the subject, e.g., in the gut.

The antibody or antigen-binding portion thereof can be administered alone or in combination with another therapeutic agent, e.g., a second monoclonal or polyclonal antibody or antigen-binding portion thereof. In one example, the antibody or antigen-binding portion thereof specifically binds to *C. difficile* toxin A is combined with a antibody (monoclonal or polyclonal) or antigen-binding portion thereof specifically binds to *C. difficile* toxin B. In another example, the second agent is an antibiotic, e.g., vancomycin, bacitracin or metronidazole. The antibodies can be used in combination

with probiotic agents such as Saccharomyces boulardii. The antibodies can also be administered in combinations with a *C. difficile* vaccine, e.g., a toxoid vaccine.

The present invention also provides compositions containing an antibody or antigen-binding portion thereof described herein, and a pharmaceutically acceptable carrier. The composition may contain an isolated nucleic acid encoding the present antibody or antigen-binding portion thereof, and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers include any and all solvents, dispersion media, isotonic and absorption delaying agents, and the like that are physiologically compatible. In one embodiment, the composition is effective to reduce, eliminate, or prevent *Clostridium difficile* bacterial infection in a subject.

The invention also features methods of treating *C. difficile* disease in a subject by administering to the subject the present antibody or antigen-binding portion thereof in an amount effective to inhibit *C. difficile* disease. Routes of administration of the present compositions include, but are not limited to, intravenous, intramuscular, subcutaneous, oral, topical, subcutaneous, intradermal, transdermal, subdermal, parenteral, rectal, spinal, or epidermal administration.

The compositions of the present invention can be prepared as injectables, either as liquid solutions or suspensions, or as solid forms which are suitable for solution or suspension in liquid vehicles prior to injection. The composition can also be prepared in solid form, emulsified or the active ingredient encapsulated in liposome vehicles or other particulate carriers used for sustained delivery. For example, the composition can be in the form of an oil emulsion, water-in-oil emulsion, water-in-oil-in-water emulsion, site-specific emulsion, long-residence emulsion, stickyemulsion, microemulsion, nanoemulsion, liposome, microparticle, microsphere, nanosphere, nanoparticle and various natural or synthetic polymers, such as nonresorbable impermeable polymers such as ethylenevinyl acetate copolymers and Hytrel® copolymers, swellable polymers such as hydrogels, or resorbable polymers such as collagen and certain polyacids or polyesters such as those used to make resorbable sutures, that allow for sustained release of the vaccine.

The present antibodies or antigen-binding portions thereof are formulated into compositions for delivery to a mammalian subject. The composition is administered

alone, and/or mixed with a pharmaceutically acceptable vehicle or excipient. Suitable vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, the vehicle can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants. The compositions of the present invention can also include ancillary substances, such as pharmacological agents, cytokines, or other biological response modifiers.

Furthermore, the compositions can be formulated into compositions in either neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the active polypeptides) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or organic acids such as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, *e.g.*, Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 21st edition.

Compositions can be administered in a single dose treatment or in multiple dose treatments on a schedule and over a time period appropriate to the age, weight and condition of the subject, the particular composition used, and the route of administration.

In one embodiment, a single dose of the composition according to the invention is administered. In other embodiments, multiple doses are administered. The frequency of administration can vary depending on any of a variety of factors, e.g., severity of the symptoms, degree of immunoprotection desired, whether the composition is used for prophylactic or curative purposes, etc. For example, in one embodiment, the composition according to the invention is administered once per month, twice per month, three times per month, every other week (qow), once per week (qw), twice per week (biw), three times per week (tiw), four times per week, five times per week, six times per week, every other day (qod), daily (qd), twice a day (qid), or three times a day (tid).

The duration of administration of a polypeptide according to the invention, e.g., the period of time over which the composition is administered, can vary, depending on

any of a variety of factors, e.g., subject response, etc. For example, the composition can be administered over a period of time ranging from about one day to about one week, from about two weeks to about four weeks, from about one month to about two months, from about two months to about four months, from about four months to about six months, from about six months to about eight months, from about eight months to about 1 year, from about 1 year to about 2 years, or from about 2 years to about 4 years, or more.

The compositions can be combined with a pharmaceutically acceptable carrier (excipient) to form a pharmacological composition. Pharmaceutically acceptable carriers can contain a physiologically acceptable compound that acts to, *e.g.*, stabilize, or increase or decrease the absorption or clearance rates of the pharmaceutical compositions of the invention. Physiologically acceptable compounds can include, *e.g.*, carbohydrates, such as glucose, sucrose, or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins, detergents, liposomal carriers, or excipients or other stabilizers and/or buffers. Other physiologically acceptable compounds include wetting agents, emulsifying agents, dispersing agents or preservatives. See *e.g.*, the 21st edition of Remington's Pharmaceutical Science, Mack Publishing Company, Easton, Pa. ("Remington's").

In one aspect, a solution of the composition are dissolved in a pharmaceutically acceptable carrier, *e.g.*, an aqueous carrier if the composition is water-soluble. Examples of aqueous solutions include, *e.g.*, water, saline, phosphate buffered saline, Hank's solution, Ringer's solution, dextrose/saline, glucose solutions and the like. The formulations can contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as buffering agents, tonicity adjusting agents, wetting agents, detergents and the like. Additives can also include additional active ingredients such as bactericidal agents, or stabilizers. For example, the solution can contain sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate or triethanolamine oleate.

Solid formulations can be used in the present invention. They can be formulated as, *e.g.*, pills, tablets, powders or capsules. For solid compositions, conventional solid carriers can be used which include, *e.g.*, mannitol, lactose, starch, magnesium stearate,

sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. Suitable pharmaceutical excipients include *e.g.*, starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol.

When administered orally, the present compositions may be protected from digestion. This can be accomplished either by complexing the antibody or antigen-binding portion thereof with a composition to render it resistant to acidic and enzymatic hydrolysis or by packaging the antibody or antigen-binding portion thereof in an appropriately resistant carrier such as a liposome. Means of protecting compounds from digestion are well known in the art. Fix, Pharm Res. 13: 1760-1764, 1996. Samanen, J. Pharm. Pharmacol. 48: 119-135, 1996. U.S. Patent No. 5,391,377.

For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated can be used in the formulation. Such penetrants are generally known in the art, and include, *e.g.*, for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents can be used to facilitate permeation. Transmucosal administration can be through nasal sprays or using suppositories. Sayani, Crit. Rev. Ther. Drug Carrier Syst. 13: 85-184, 1996. For topical, transdermal administration, the agents are formulated into ointments, creams, salves, powders and gels. Transdermal delivery systems can also include, e.g., patches.

The present compositions can also be administered in sustained delivery or sustained release mechanisms. For example, biodegradeable microspheres or capsules or other biodegradeable polymer configurations capable of sustained delivery of a peptide can be included in the formulations of the invention (see, e.g., Putney, Nat. Biotechnol. 16: 153-157, 1998).

For inhalation, the present compositions can be delivered using any system known in the art, including dry powder aerosols, liquids delivery systems, air jet nebulizers, propellant systems, and the like. Patton, Biotechniques 16: 141-143, 1998. Also can be used in the present invention are product and inhalation delivery systems for polypeptide macromolecules by, *e.g.*, Dura Pharmaceuticals (San Diego, Calif.), Aradigrn (Hayward, Calif.), Aerogen (Santa Clara, Calif.), Inhale Therapeutic Systems (San Carlos, Calif.),

and the like. For example, the pharmaceutical formulation can be administered in the form of an aerosol or mist. For aerosol administration, the formulation can be supplied in finely divided form along with a surfactant and propellant. In another aspect, the device for delivering the formulation to respiratory tissue is an inhaler in which the formulation vaporizes. Other liquid delivery systems include, *e.g.*, air jet nebulizers.

Compositions or nucleic acids, polypeptides, or antibodies of the invention can be delivered alone or as pharmaceutical compositions by any means known in the art, *e.g.*, systemically, regionally, or locally; by intra-arterial, intrathecal (IT), intravenous (IV), parenteral, intra-pleural cavity, topical, oral, or local administration, as subcutaneous, intra-tracheal (*e.g.*, by aerosol) or transmucosal (*e.g.*, buccal, bladder, vaginal, uterine, rectal, nasal mucosa). For a "regional effect," *e.g.*, to focus on a specific organ, one mode of administration includes intra-arterial or intrathecal (IT) injections, *e.g.*, to focus on a specific organ, *e.g.*, brain and CNS (see e.g., Gurun, Anesth Analg. 85: 317-323, 1997). For example, intra-carotid artery injection can be used where it is desired to deliver a nucleic acid, peptide or polypeptide of the invention directly to the brain. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in detail. Bai, J. Neuroimmunol. 80: 65-75, 1997. Warren, J. Neurol. Sci. 152: 31-38, 1997. Tonegawa, J. Exp. Med. 186: 507-515, 1997.

In one aspect, the pharmaceutical formulations comprising compositions or nucleic acids, polypeptides, or antibodies of the invention are incorporated in lipid monolayers or bilayers, e.g., liposomes. U.S. Patent Nos. 6,110,490; 6,096,716; 5,283,185 and 5,279,833. Aspects of the invention also provide formulations in which water soluble nucleic acids, peptides or polypeptides of the invention have been attached to the surface of the monolayer or bilayer. For example, peptides can be attached to hydrazide-PEG-(distearoylphosphatidyl) ethanolamine-containing liposomes (see, *e.g.*, Zalipsky, Bioconjug. Chem. 6: 705-708, 1995). Liposomes or any form of lipid membrane, such as planar lipid membranes or the cell membrane of an intact cell, *e.g.*, a red blood cell, can be used. Liposomal formulations can be by any means, including administration intravenously, transdermally (see, e.g., Vutla, J. Pharm. Sci. 85: 5-8, 1996), transmucosally, or orally. The invention also provides pharmaceutical preparations in which the nucleic acid, peptides and/or polypeptides of the invention are incorporated

within micelles and/or liposomes (see, e.g., Suntres, J. Pharm. Pharmacol. 46: 23-28, 1994; Woodle, Pharm. Res. 9: 260-265, 1992). Liposomes and liposomal formulations can be prepared according to standard methods and are also well known in the art. Akimaru, Cytokines Mol. Ther. 1: 197-210, 1995. Alving, Immunol. Rev. 145: 5-31, 1995. Szoka, Ann. Rev. Biophys. Bioeng. 9: 467, 1980. U.S. Patent Nos. 4, 235,871; 4,501,728 and 4,837,028.

In one aspect, the compositions are prepared with carriers that will protect the peptide against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. U.S. Patent No. 4,522,811.

It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. In one embodiment, the dosage of such compounds lies within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. In another embodiment, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Sonderstrup, Springer, Sem. Immunopathol. 25: 35-45, 2003. Nikula *et al.*, Inhal. Toxicol. 4(12): 123-53, 2000.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antigen-binding portion of the invention is from about

0.001 to about 60 mg/kg body weight, about 0.01 to about 30 mg/kg body weight, about 0.01 to about 25 mg/kg body weight, about 0.5 to about 25 mg/kg body weight, about 0.1 to about 20 mg/kg body weight, about 10 to about 20 mg/kg body weight, about 0.75 to about 10 mg/kg body weight, about 1 to about 10 mg/kg body weight, about 2 to about 9 mg/kg body weight, about 1 to about 2 mg/kg body weight,about 3 to about 8 mg/kg body weight, about 4 to about 7 mg/kg body weight, about 5 to about 6 mg/kg body weight, about 8 to about 13 mg/kg body weight, about 8.3 to about 12.5 mg/kg body weight, about 4 to about 6 mg/kg body weight, about 4.2 to about 6.3 mg/kg body weight, about 1.6 to about 2.5 mg/kg body weight, about 2 to about 3 mg/kg body weight, or about 10 mg/kg body weight.

The composition is formulated to contain an effective amount of the present antibody or antigen-binding portion thereof, wherein the amount depends on the animal to be treated and the condition to be treated. In one embodiment, the present antibody or antigen-binding portion thereof is administered at a dose ranging from about 0.01 mg to about 10 g, from about 0.1 mg to about 9 g, from about 1 mg to about 8 g, from about 1 mg to about 7 g, from about 5 mg to about 6 g, from about 10 mg to about 5 g, from about 20 mg to about 1 g, from about 50 mg to about 800 mg, from about 100 mg to about 500 mg, from about 0.01mg to about 10 g, from about 0.05 μg to about 1.5 mg, from about 10 μg to about 1 mg protein, from about 30 μg to about 500 μg, from about 40 pg to about 300 pg, from about 0.1 μg to about 200 mg, from about 0.1 μg to about 5 μg, from about 5 μg to about 10 μg, from about 10 μg to about 25 μg, from about 25 μg to about 50 μg, from about 50 μg to about 100 μg, from about 100 μg to about 500 μg, from about 500 μg to about 1 mg, from about 1 mg to about 2 mg. The specific dose level for any particular subject depends upon a variety of factors including the activity of the specific peptide, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

In therapeutic applications, the present compositions are administered to a subject at risk for *Clostridium difficile* bacterial infection or suffering from active infection in an amount sufficient to at least partially arrest or prevent the condition or a disease and/or its complications.

An anti-toxin antibody (e.g., monoclonal antibody) can also be used to isolate toxins by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an anti-toxin antibody can be used to detect the toxin, e.g., to screen samples (e.g., in a stool sample) for the presence of *C. difficile*. Anti-toxin antibodies can be used diagnostically to monitor levels of the toxin in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen.

The invention also provides kits containing an anti-toxin antibody or antigen-binding portion thereof. Additional components of the kits may include one or more of the following: instructions for use; other reagents, a therapeutic agent, or an agent useful for coupling an antibody to a label or therapeutic agent, or other materials for preparing the antibody for administration; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject.

Various combinations of antibodies can be packaged together. For example, a kit can include antibodies that bind to toxin A and antibodies that bind to toxin B (e.g., monoclonal anti-toxin B antibodies, or polyclonal antisera reactive with toxin B). The antibodies can be mixed together, or packaged separately within the kit.

Instructions for use can include instructions for therapeutic application including suggested dosages and/or modes of administration, e.g., in a patient with a symptom of CDAD. Other instructions can include instructions on coupling of the antibody to a label or a therapeutic agent, or for purification of a conjugated antibody, e.g., from unreacted conjugation components.

The kit may or may not contain at least one nucleic acid encoding anti-toxin antibodies or fragment thereof, and instructions for expression of the nucleic acids. Other possible components of the kit include expression vectors and cells.

The present antibodies, antigen-binding portions thereof, compositions and methods can be used in all vertebrates, *e.g.*, mammals and non-mammals, including human, mice, rats, guinea pigs, hamsters, dogs, cats, cows, horses, goats, sheep, pigs, monkeys, apes, gorillas, chimpanzees, rabbits, ducks, geese, chickens, amphibians, reptiles and other animals.

The following examples of specific aspects for carrying out the present invention are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

EXAMPLES

Example 1: Hybridoma Fusion

A classical hybridoma fusion was performed. Mice receive their first immunization with toxoid A using Complete Freund's Adjuvant (CFA) and two subsequent boosters on days 28 and 48 with toxoid A and Incomplete Freund's Adjuvant (IFA). A trial bleed was performed at day 55 and the serum was tested to check for titres of anti-toxoid A antibody. If IgG titres were high enough fusions were performed. If not, mice received two more boosts with IFA and a second trial bleed was taken. Fusions were performed using 2 mice at a time. Mice were given a final push intraperitoneally (i.p.) with toxoid A in PBS three days prior to the fusion.

On the day of the fusion, mice are sacrificed and their spleens removed. Splenocytes are washed from the spleen using a syringe and needle and collected in a 50 ml tube for fusion with myeloma cells. Myelomas are an immortal tumor cell line used as fusion partners, grown in the presence of 8-azaguanine, a toxic nucleotide analog which blocks the salvage pathway. Cells grown in the presence of 8-aza survive only by incurring defective mutations in the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) gene. B cells are fused with the myeloma cells using Polyethylene Glycol (PEG) 1500. Fused cells are mixed into semi-solid agarose with drug selection and plated out into petri dishes. HAT media containing Hypoxanthine, Aminopterin, and Thymidine is used for drug selection. Aminopterin is a drug which inhibits the de novo pathway for nucleotide metabolism which is absolutely required for survival/cell growth in myeloma lines defective in HGPRT, and allows selection usually within 24-48 hours.

Example 2: Hybridoma Screening

The next step is screening of the growing hybridomas. A commercial semisolid agarose within which the cells grow as "balls" of cells in the 3-D matrix was used. This facilitates the picking of these balls by hand (by visual inspection) and transferring these

clonal balls into a 96 well plate containing suitable media. The cells were allowed to grow for 3-7 days and then the supernatant removed for screening and replaced with fresh media. Positive binding in ELISA (or other tests) resulted in continuing to grow the hybridomas by transferring them up into larger tissue culture vessels with increasing volume. The mAbs were isotyped using a suitable commercial isotyping kit for murine mAbs using the spent supernatant. The decision to move a clone to the next stage of selection is based on its reactivity to native toxin A using an ELISA and its survival, usually based upon serial dilutions and reactivity of at least 1/8 or 1/16 or higher, as well as IgG class; therefore the number of clones decreased throughout the selection procedure. The mAbs that underwent further characterization were: CAN20G2, CAN20G1, CAN20G5 & CAN20G8 and CAN19G1, CAN19G2, CAN19G3.

Example 3: ELISA Assay of Mouse Monoclonal Antibodies

An ELISA was used to test the binding of the toxA mAbs against whole toxin A and recombinant toxin A fragment 4 as well as to determine if they were cross-reactive to whole toxin B and toxin B fragment 4. The mAb clones were compared to CDA1 (Merck anti-toxin A mAb used as a control). The ELISA plate was coated with 100 μg/ml of Toxin fragment 4 and 400 μg/ml of whole Toxin so that the coatings were equimolar. The wells were blocked with 1% skim milk then probed with serially diluted CAN19 or CAN20 mAbs (0.1 μg/ml to1 μg/ml) and binding was detected with a commercial goat anti-mouse IgG-HRP antibody. Negative and positive controls were also run. The chimeric human mAb 13C6 is specific to Ebolavirus GP and served as the negative control for human the Fc of CDA-1. The CDA-1 mAb and the polyclonal toxoid A antibody (pAb) served as positive controls, however, the CDA-1 mAb is human and the polyclonal is rabbit, thus, they both used a different secondary antibody making direct comparisons between them and the murine mAbs impossible. The secondary antibody control is for the murine secondary antibody. The plate was read at 405 nm after 60 min incubation with substrate. The titration data for each antibody is shown in Figure 1.

Results: As shown in Figures 2 and 3, CAN19G1 and CAN19G2 mAbs bind to whole toxin A and toxin A fragment 4 at a similar level to CDA1. The CAN19 mAbs showed little cross-reactivity to toxin B. CAN20 mAbs bind to toxin A. CAN20G2 and

CAN20G5 bind to toxin A fragment 4 at a similar level to CDA1. None of CAN20 mAbs showed cross-reactivity to toxin B.

Example 4: Western Blot of Mouse Monoclonal Antibodies

A 4-12% SDS-PAGE gel was run for 1.5 hours at 200 volts with a combination of *C.difficile* proteins; whole toxin A, toxoid A (commercial), recombinant toxin fragment 4 and toxin B (whole, toxoid and fragment 4). The gel was then transferred to a nitrocellulose membrane for 45 min at 45 volts. The membrane was blocked overnight at 4°C with 1% skim milk in 1xTBST and the next day washed with 1xTBST to remove the skim milk. The mAbs (1° Ab) were diluted in 1xTBST at a concentration of 1µg/ml and used to probe the membrane containing the transferred products for 2 hours at room temperature (RT) on a shaker. The membranes were then washed with 1xTBST to remove unbound 1° Ab and probed with anti-mouse IgG-HRP (2° Ab) at a dilution of 1:4000 for 1 hour at RT on a shaker.

Results: As shown in Figures 4 and 5, all three CAN19 mAbs showed binding to whole toxin A, toxoid A and toxin A fragment 4. They all showed only weak or no cross-reactivity to toxin B or to the negative control. CAN20G1, CAN20G2, CAN20G5 and CAN20G8 mAbs all showed strong binding to whole toxin A and toxin A fragment 4. There was no cross-reactivity to toxin B or to the negative control.

Example 5: Affinity Analysis of Mouse Monoclonal Antibodies

Biolayer interferometry was used to measure the interactions between whole Toxin A and the anti-toxin A antibodies. The Octet QKe instrument (ForteBio) was equipped with Streptavidin (SA) biosensors. 40µg/ml of biotinylated whole Toxin A was coupled to SA sensors and the toxin A mAbs, in a dilution series from 100 nM to 1.56 nM, were reacted on the toxin-coated pins for 10 minutes followed by a dissociation step in PBS for another 10 minutes. The results were then analyzed using ForteBio Data Analysis software to determine the dissociation constant (K_D), which is the measure used to describe the binding strength between antibody and antigen, k_{on}(1/Ms), the on-rate at which antibody antigen complexes form, and k_{dis}(1/s), the off-rate at which the antibody

antigen complexes dissociate. The samples were run over two separate days. Table 4 shows affinity data for purified CAN20G versions, as well as CDA1.

Table 4 Affinity data for purified CAN20G versions and CDA1

ID Name	K_D (M)	k_{on}(1/Ms)	k_{dis} (1/s)
CAN20G1	1.79E-09	1.23E+05	2.19E-04
CAN20G2	4.19E-12	1.04E+05	4.35E-07
CAN20G5	2.01E-09	8.38E+04	1.68E-04
CAN20G8	1.65E-09	1.31E+05	2.16E-04
CDA1	6.24E-10	4.80E+05	2.91E-04

Example 6: Epitope Binning of Mouse Monoclonal Antibodies

The Octet QKe is a label free real-time biosensor that uses disposable fiber-optic sensors that detect biomolecular interactions via biolayer interferometry. The epitope binning assay was performed against the previously characterized CDA1 anti-toxin A mAb to examine whether the present toxin A mAbs share a similar or a different epitope with CDA-1. Secondly, the assay was used to confirm shared single or potentially multiple epitope bins between the toxin A mAbs. The classical sandwich method was used and involves coupling the mAb to sensor, binding antigen, and then binding to another mAb. The second mAb can bind the captured Ag only if its epitope does not overlap that of the immobilized mAb.

Results: The strong nM shift in wavelength above the CAN20G1 and PBS control (a vertical increase in the binding curve) indicates more binding is able to occur and that the test antibody is binding to an exposed and distinct epitope. As shown in Figures 6a and 6b, the results indicate that there is an elevated shift in wavelength for the CDA1 antibody. This indicates that the CDA1 and CAN20 mAbs bind to distinct epitopes. All the CAN20 mAbs share the same epitope. There is a slight nm elevation for the CAN20G2 indicating a slight increase in binding which could be due to a somatic mutation between the known VH and VL chains of CAN20G1 and CAN20G2, different antibody epitopes or both.

Example 7: In vitro neutralization of Mouse Monoclonal Antibodies

The *in vitro* neutralization assays described herein were performed using VERO (green monkey) cells and Toxin A purchased from List Biological Laboratories. (BIAD report: *Clostridium difficile* Toxin A Monoclonal Antibody Characterization). The protocols used for xCelligence (Roche Diagnostics) and Bioassy methods are summarized below.

Cell attachment Phase – xCelligence Method. (1) Trypsinized cells in source flask. (2) Added 2 mL of trypsin to flask and washed cells to remove traces of media then aspirate. (3) Added 3 mL of trypsin and incubated at 37°C for approximately 10 minutes until cells were detached. (4) Added 6 mL of assay media or growth media to flask. (4) Centrifuged at 1300 rpm for 8 minutes. (5) Aspirated supernatant and resuspended cells with 6 mL of Assay media or growth media. (6) Counted cells and calculated required cell density. (For Vero cells, $1x10^5$ cells/mL and for T84 cells, 8×10^5 cells/mL.) (7) To a 96 well E-plate added 100 µL of Assay media to wells A1 thru H10 and 100 µL of T84 media to wells A11 thru H12. (8) Performed background reading on xCelligence. (9) Removed 50 µL of Assay media from wells A1 – H10. (10) Added 50 µL of $1.0x10^5$ cells/mL suspension to these wells for a final $5.0x10^4$ cells/mL seeding density. (11) Added 100 µL of T84 $8x10^5$ cells/mL cell suspension to A11 and A12. (12) Serially diluted 2-fold down through H11 and H12. (13) Remove 100 µL from H11 and H12. (14) Added 100 µL of T84 media to A11 – H12 for a final volume 200 µL. (15) Incubated plate at room temperature for 20 – 30 minutes to allow cells to settle evenly. (16) Placed plate in 37°C incubator with 5% CO2 overlay for 20 – 24 hours.

Cell attachment phase – Bioassy method. (1) Trypsinized cells in source flasks. (2) Pooled cells from source flasks. (3) Centrifuged cells at 1270 RPM for 8 minutes. (4) Removed supernatant and resuspended cells in assay medium. (5) Six mL of medium should be used for every flask pooled. (6) Counted cells to determine cell viability and quantity of cells required to plate at 1.0×10^5 cells/mL. (7) Final concentration will be 0.5×10^5 cells/mL when plated. (8) Added 50 µL of 10% Assay Media to wells B2 – G11 of a 96 well black clear-bottom microplate. (9) Overlayed 50 µL of cells to wells B2 – G11 of a 96 well flat-bottom microplate at $1.0x10^5$ cells/mL. (10) To the outer edge wells, added 100 µL of warmed assay media. (11) Mixed on a plate shaker for a homogeneous suspension. (12) Left plate at room temperature for 20 – 30 minutes to allow cells to

settle evenly across the wells. (13) Placed cell plates in a 37°C, 5% CO_2 humidified incubator for 20-24 hours.

Toxin A preparation: (1) Prepared Toxin A primary stock (20 μg/mL) by adding 100 μL of sterile LW to one vial (2.0 μg) of Toxin A. (2) Diluted primary stock as shown in Table 5.

Table 5

TcdA Concentration (ng/mL)	Final Plating Concentration (ng/mL)	Volume of TcdA Primary Stock (20 μg/mL)	Volume of 10% Assay Medium
60	20	12 μL	3988 μL

Sample Preparation: To test potency, all the monoclonal antibodies were at a starting concentration of 30 μg/mL. Samples were prepared as shown in Table 6.

Table 6

Sample (Stock concentration)	**Preparation Concentration**	**Final Plating Concentration**	**Volume Sample Stock**	**Volume Assay Medium**
CDA (1.556 mg/mL)	30 μg/mL	10 μg/mL	28.9 μL	1471.1 μL
CAN19 G1 Purified 30 μg/mL	30 μg/mL	10 μg/mL	150 μL / plate	n/a
CAN19 G2 Purified 30 μg/mL	30 μg/mL	10 μg/mL	150 μL / plate	n/a
CAN19 G3 Purified 30 μg/mL	30 μg/mL	10 μg/mL	150 μL / plate	n/a

Dilution plate preparation-xCelligence: (1) Added assay media and 150 μL of sample to wells as shown below in Table 7. (2) Serially diluted each sample 2-fold down the column by taking 75 μL from Row A and adding to Row B, mixed 3 to five times and repeated down through to Row G. (3) Added appropriate controls to wells as shown in

Table 5. (4) Overlayed sample wells with 75 µL of Toxin A.Shake on a plate shaker until homogeneous. (5) Incubated at 37°C for 1 hour.

Table 7: xCelligence Dilution Plate Layout

Cell Density: 0.5e5 cells/mL												
CDA	CDA	G1	G1	G2	G2	G3	G3	G1 Sup	G1 Sup	G2 Sup	G2 Sup	G2 Sup
1	2	3	4	5	6	7	8	9	10	11	12	
A Sample	Sample	Sample	Sample	Sample	Sample	CDA Ctl	CDA Ctl	Sample	Sample	150 uL	150 uL	
B 75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	150 uL	150 uL	
C 75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	150 uL	150 uL	
D 75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	150 uL	150 uL	
E 75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	150 uL	150 uL	
F 75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	150 uL	150 uL	
G 75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	75 uL	Cell Ctl	Cell Ctl	Tox Ctl	Tox Ctl	
H CDA Ctl	CDA Ctl	G1 Ctl	G1 Ctl	G2 Ctl	G2 Ctl	G3 Ctl	G3 Ctl	G1 Sup Ctl	G1 Sup Ctl	G2 Sup Ctl	G2 Sup Ctl	
	Cell control = 150 µL AM					75 uL / 150 uL = volume AM						
	Toxin control = 75 µL toxin + 75 µL AM											
	mAb control = 75 µL mAb + 75 µL AM					Sample = 150 uL respective sample						

Bioassay method: Added assay medium to wells as shown in Table 8.

(1) Add 150 µL of sample to appropriate wells of column 2. On plate #1, add CDA, CAN19G1 (purified), and CAN19G1 supernatant. On plate #2 added CDA, CAN19G2

purified, and CAN19G2 supernatant. On plate #3 added CDA, CAN19G3 purified, and CAN19G3 supernatant. (2) Transferred 75 µL from column 2 to column 3. Mixed with multichannel. Repeated procedure through to column 9. (3) Remove 75 µL from column 10 leaving a final volume 100 µL. (4) Added controls (75 µL) to appropriate wells along with 75 µL of AM. (5) Overlayed sample wells with 75 µL of Toxin A.

Table 8: Bioasssay Dilution Plate Layout

Cell Density: 0.5e5 cells/mL												
		50 µL Serial Dilutions									Controls	
	1	2	3	4	5	6	7	8	9	10	11	12
A	200 µL AN	200 µL AM	200 µL AN	200 µL AN	200 µL AN	200 µL AN	200 µL AN	200 µL AN	200 µL AN	200 µL AN	200 µL AN	200 µL AN
B	200 µL AN	CDA	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	CDA ctl	Cell	200 µL AN
C	200 µL AN	CNJ mAb	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	mAb Ctl	Toxin	200 µL AN
D	200 µL AN	CNJ sup	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	sup Ctl	Cell	200 µL AN
E	200 µL AN	CDA	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	CDA ctl	Toxin	200 µL AN
F	200 µL AN	CNJ mAb	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	mAb Ctl	mAb	200 µL AN
G	200 µL AN	CNJ sup	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	75 µL AM	sup Ctl	pAb	200 µL AN
H	200 µL AN	200 µL AM	200 µL AN	200 µL AN	200 µL AN	200 µL AN	200 µL AN	200 µL AN	200 µL AN	200 µL AN	200 µL AN	200 µL AN
		Cell control = 150 µL AM										
		Toxin control = 75 µL toxin + 75 µL AM										
		mAb control = 75 µL mAb + 75 µL AM										

Sample addition to cell plates: (1) Following 1 hour incubation, cell plates were removed from incubator. (2) Removed 50 µL of cell suspension carefully with multichannel pipette being sure not to disturb cell monolayer. (3) Transfered 100 µL of samples from dilution plate to appropriate wells of cell plate. (4) Mixed on plate shaker for a homogeneous solution. Incubated 72 hours at 37°C with a 5% CO2 overlay.

Data analysis: The xCelligence system captures data in real-time. For the purposes of comparison to the conventional bioassay methods, the final read time data is analyzed. For this, we normalized the cell index at the time point before toxin / antibody addition to the plate, using the appropriate toxin wells as baseline. This will create a baseline normalized cell index on the Y axis versus log concentration of antibody.
We analyzed the data to determine potency of CAN19 mAbs in comparison to CDA.
% Neutralization is calculated as follows with xCelligence:
% Neutralization = (Sample CI index / Antibody Control CI index) * 100
% Neutralization is calculated as follows with Bioassay fluorescence:
% Neutralization = (Mean Sample RFU/ Mean Toxin RFU)/ (Mean Cell RFU/ Mean Toxin RFU)*100
The procedures of this Example were also performed on CAN20 mAbs.

Results: CAN19 mAbs were less neutralizing than CDA1. CAN20G2 is the most potent mAb *in vitro* and is more potent than CDA1. CAN20G3, G5 and G8 are also neutralizing.

Table 9 summarizes the IC_{50} data generated for each CAN19 mAb demonstrating that the CAN19 clones are less neutralizing compared to CDA1.

Table 9

Sample	IC50	
CDA	0.347 ug/mL	standard
1	2.31 ug/mL	CAN19G1
2	2.17 ug/mL	CAN19G2
3	2.44 ug/mL	CAN19G3

Table 10 summarizes the EC_{50} data generated for each CAN20 mAb demonstrating that CAN20G2, CAN20G3, CAN20G5, and CAN20G8 are the most neutralizing of the clones.

Table 10

ID Name	Calculated anti-TcdA IgG Concentration By Biacore(μg/mL)	EC50 Value (μg/mL)[1]
CAN20G1	188.0	0.17
CAN20G2	142.2	0.0101
CAN20G3	5.9	0.076
CAN20G4	22.8	0.147
CAN20G5	87.5	0.13
CAN20G6	314.2	0.151
CAN20G7	134.9	0.158
CAN20G8	272.4	0.137

[1]The EC50 value is the concentration of antibody which neutralizes 50% of the TcdA toxin dose.

Example 8: Mouse in vivo toxin challenge

The mouse *in vivo* toxin challenge test was based on previous publications (Babcock *et al.*, Human Monoclonal Antibodies Directed against Toxins A and B prevent *Clostridium difficile*-Induced Mortality in Hamsters. Infection and Immunity (2006) 74(11):6339). Swiss webster mice weighing 20-30 g were given 250 μg of mAb or controls at day 0 and allowed to rest. After 24 hrs (day 1), the mice were given a lethal dose of TcdA (100 ng). This dose kills 90-100% of animals by 24 hours in an unprotected state. The mice were observed for 7 days (days 1 – 7) for signs of abnormality and local and systemic disease. The mice were euthanized on Day 7. All observations were recorded and the % survival was determined for each treatment group.

Results: As shown in Figures 7, 8, and 9, the study results indicate that the CAN19 and CAN20 mAbs protect mice against toxin A. There was > 90% survival with CAN19G1, G2 and G3. All three CAN19 mAbs showed efficacy. All the CAN20 mAbs were efficacious. CAN20G1, G2, G5 and G8 showed 100% protection at a dose of 0.25 mg/mouse. CAN20G2 showed 100% protection at 0.125 mg/mouse. The experiment was repeated to confirm the efficacy of CAN20G2. The results confirmed the previous study.

CAN20G2 showed 100% protection at the full does of 0.25 mg/mouse and 90% protection at the half dose 0.125 mg/mouse.

Example 9: muCAN20G2 V Gene Sequencing

RNA was isolated from the CAN20G2 parental hybridoma clonal cell line using the RNeasy Mini Kit. The amplification of V genes from the RNA was performed using the Qiagen OneStep RT-PCR Kit. Several combinations of primer sets were used as follows: for immunoglobulin variable region gene sequence confirmation from the hybridomas, a set of Variable region gene (V-gene) subgroup-specific oligonucleotide primers are used. These include 5'mVK-Lead-1, 3'KappaConstRT, 5'mVH-Lead-2, 5'mVH-Lead-2A, and 3'mIG1-2C RT. In order to rule out potential contamination from the known and endogenous aberrant kappa light chain V-gene mRNA (found within P3X63 myelomas) (Yuan, X. *et al.*, J. Immunol. Methods, 294: 199-207 (2004)), the RT-PCR was also performed using non-subgroup specific primer sets, 5'mVK-Lead-1A, 5'mVK-Lead-1A, 5'mVK-Lead-3, 5'mVK-Lead-3A, 5'mVH-IGHV1-Lead, 5'mVH-Lead-1, 5'mVH-Lead-3, 5'mVH-Lead-4, and 5'mVH-Lead-5. Refer to Figure 10 for a list of the primers and their sequences. The results of the PCR amplification reactions were determined by examining the PCR products on an analytical agarose gel, and the visualized bands at approximately 500bp were gel isolated for cloning. The extracted DNA was directly TA cloned into the pCR2.1-TOPO vector using the low melt agarose method in the TOPO TA Cloning manual. Five colonies of each CAN20G clone reaction were sequenced in both directions using the M13 Forward and M13 Reverse primers. Sequence data was analyzed using DNAStar Lasergene software. The resulting rearranged V-gene sequences were compared to IMGT/V-Quest reference directory sets and to the NCBI immunoglobulin blast search (Figure 11).

Example 10: Humanization of muCAN20G2

Three humanized IgG/k versions of CAN20G2 mAb have been created as well as a chimeric IgG1/k. For the humanized versions, maximum identity alignment with human germline alleles was used (from the IMGT and NCBI websites) to help to identify acceptor frameworks. All 6 CDRs were inserted. Other residues were changed

or maintained due to surface exposure or involvement in folding or interchain contacts, respectively. The CDRs of the murine mAb sequence (CAN20G2) match very well with the germline CDRs of the closest human alleles. This resembles the "superhumanization" approach where CDR matching rather than total framework is used in a variation of the use of germline sequences as acceptor frameworks. In the case of Tan *et al.*, J. Immunol. 2002, 169:1119-1125, the authors used the CDR sequences and tried to match the so called canonical classes of CDRs based upon the Chothia classification system. However, because particular CDRs are germline encoded and particular canonical conformations tend to be found in certain frameworks, the "Superhumanization" method of choosing acceptor frameworks does not in all cases result in the selection of a different candidate acceptor framework. It is empirical and remains to be tested for multiple mAb specificities. This is in part because the straight-up alignment of frameworks for identity inherently encompasses the CDRs as well in the comparison. Table 11 shows the percent humanness, at the amino acid level, of each of the humanized constructs of CAN20G2.

Table 11

CONSTRUCT	PERCENT "HUMAN"
MURINE CAN20G2	66%
CHIMERIC CAN20G2	90%
HE-CAN20G2	91%
hCDR-CAN20G2	97%
AVA-CAN20G2	95%

Figure 12 shows the alignment of muCAN20G2 v-regions with the closest human germline v-region. The human germlines were used as acceptor frameworks for humanization.

CDR-huCAN20G2 – CDR Grafted only. The best matching germline allele for both V_H and Vk were used as an acceptor framework for grafting the CDRs. No other changes were made to the acceptor frameworks. Figures 13a and 13b show the design of the CDR-huCAN20G2 design we used. The closest matching human frameworks are IGHV7-4-1*02 and IGKV1-39*01. The CDRs (IMGT Numbering) of the muCAN20G2

were inserted into the human framework. The heavy CDR3 contained a HpaI restriction site that was altered for cloning into pcDNA3002Neo. A 5' Kozak and HAVT20 leader sequence was added for correct translation and trafficking.

HE-huCAN20G2 "Human engineered" This humanized version was generated using a strategy most similar to the "human engineering" strategy used by Studnicka *et al* (1994) used to humanize a murine mAb to CD5. Essentially, the closest human germline allele for both CAN20G2 V_H and Vk were identified, individually, and designed for use as acceptor frameworks. The CAN20G2 V_H has a 76% identity with the human IgVH7-4-1*02 allele. The CDRs were grafted or altered to match the CAN20G2 mAb sequences. The HE-hCAN20G2 antibodies are shown in Figures 14, 15, and 16. Some residues were modified or maintained as described in the legend. In this case, crystal structural inference was taken from Avastin / Bevacizumab. Avastin is a humanized monoclonal antibody that recognizes and blocks vascular endothelial growth factor A (VEGF-A) and is marketed for the treatment of advanced colorectal cancer. Avastin turns out to have highest identity with the same human germline gene as CAN20G2 VH and the crystal structure of its variable region structure has been determined.

AVA-huCAN20G2 "Avastinized" – Alignment of the translation of the Avastin V_H and Vκ/Jκ alleles with the respective humanized CAN20G2 V_H and Vκ immunoglobulin variable regions is shown in Figure 17. Many mAbs have been humanized capitalizing on the natural sequence pairing of V_H and V_L found in other mAbs with crystal structural data. In this case, we used the same V_H as in Version 1 – HE (which has high identity with Avastin V_H), and we used the Avastin Vk as the Light chain acceptor framework. This allowed us to exploit the known interchain contacts and modification in our design (Figure 18).

Chimeric-huCAN20G2 Chimeric Version: A chimeric CAN20G2 was designed as a control. Certain residues outside the CDRs are involved in the structure of the hypervariable regions. During the humanization process some of the residues may be altered. Because sequence variation within the canonical structures will modulate the conformation of the paratope, it is essential to determine whether the loss/gain in affinity/function/neutralization is due to the humanization process or the human Fc

region. The CAN20G2 murine v-regions were designed onto human IgG1 and human Kappa constant regions. The construct contains Kozak, HAVT20 Leader and double stop sequences (Figures 19A and 19B).

Example 11: SDS Page and Western Blot Analyses of Humanized Antibodies

A large scale transfection (300ml) was performed in HEK293F cells to obtain a large quantity of each huCAN20G2 mAb. A total of $3x10^8$ cells were transfected with 300 μg of huCAN20G2 plasmid DNA. The supernatant was harvested by centrifugation (3000 rpm, 15 min, RT) 3 days and 7 days post-transfection. The transfected supernatant was filtered through a 0.22 μm filter. The filtered sup was purified on a Protein G column (HiTRAP HP, GE Healthcare) using the AktaPurifier FPLC. The eluted protein was buffer exchanged into D-PBS and the concentration determined by BCA assay. A range of 30-45 mg was purified from the 300 ml cultures. The purified protein was run on an SDS-page to confirm its size (Figure 22). The purified mAb was also used to probe a membrane with whole toxin A and toxin A fragment 4 to confirm the binding characteristics of the mAbs (Figure 23).

Example 12: In vitro Neutralization Assay of Humanized Antibodies

An *in vitro* neutralization assay for *C.difficile* Toxins using CT-26 cells was performed to test the neutralization capability of the humanized mAb clones against *C. difficile* toxin A. The CT-26 cells were seeded in a 96 well plate at a concentration of 2.5-$3x10^4$ cells/100ul/well and the plate was incubated in a CO_2 incubator for 4-5 hrs at 37°C. Two blank wells containing only media (no cells) were also included in the plate.

The toxin and toxin/Ab mixtures were prepared in tubes and diluted to the desired concentrations using RPMI media. The tubes were left to incubate at room temperature for 1 hour. The media was removed from the wells of the plate and each of the tubes, containing either media alone, toxin alone, or toxin/Ab mixtures, was transferred to its designated well. The plates were left to incubate for 48 hours at 37°C and 5% CO_2. The WST-1 detection reagent was added to each well (10 μl of reagent/100 μl volume in the well) and incubated for 1 hour at 37°C and 5% CO_2. The plate was shaken for 1 min and then read at 450 nm.

Cell viability was determined based on the cell controls as below:

% Cell viability =Mean OD of test/MeanOD of cell control x 100.

Toxin neutralization is calculated by the formula as below:

% Neutralization = (Sample OD – Toxin control OD)/ (Cell control OD – toxin control OD)*100

Results: As shown in Figures 20a and 20b, the chimeric CAN20G2 and the HE-CAN20G2 are the most neutralizing at all mAb concentrations. The HE-CAN20G2 is more neutralizing at most mAb concentrations at either Toxin A concentration. The Medarex CDA IgG and the hCDR mAbs show similar modest neutralization ability and the AVA-CAN20G2 shows very little neutralization ability.

Example 13: Affinity Assay of Humanized Antibodies

Biolayer interferometry was used to measure the interactions between whole Toxin A and the humanized CAN20G2 antibodies. The Octet QKe instrument was equipped with Streptavidin (SA) biosensors. 40 μg/ml of biotinylated whole Toxin A was coupled to SA sensors and the humanized versions, in a dilution series from 100nM to 1.56nM, was allowed to associate with the toxin for 10 minutes followed by a dissociation step in PBS for another 10 minutes. The results were then analyzed using ForteBio Data Analysis software to determine K_D (nM), the measure used to describe the binding strength between antibody and antigen, k_{on}(1/Ms), the rate at which antibody antigen complexes form, and k_{dis}(1/s), the rate at which the antibody antigen complexes dissociate.

Results: The results from two experiments were averaged and show that the muCAN20G2 and the chCAN20G2 are within threefold indicating no loss in affinity (Table 10). In contrast, the AVA-CAN20G2 showed almost a full log loss in affinity. The CDR-huCAN20G2 showed loss in affinity nearing that of the AVA humanized version. The binding affinity of the HE-huCAN20G2 version is slightly higher than all the other humanized versions but within the acceptable threefold range showing little or no loss of

affinity compared to the chimeric CAN20G2. We believe this is the optimal comparator because we cannot predict the effects of exchanging the human constant regions for the murine IgG2a constant regions and this comparison takes this into account. The three fold range comparison is considered by the ForteBio experts as insiginificant variation.

Table 12 Affinity data for purified human CAN20G2 versions.

	K_D(M)	k_{on}(1/Ms)	k_{dis}(1/s)
muCAN20G2-2-1	1.66E-10	1.08E+05	1.80E-05
chCAN20G2	1.72E-10	1.14E+05	1.93E-05
AVA-CAN20G2	1.33E-09	5.45E+04	9.02E-05
HE-huCAN20G2	3.32E-10	9.39E+04	3.14E-05
CDR-huCAN20G2	8.00E-10	6.76E+04	5.41E-05

Example 14: ELISA Testing of Humanized Antibodies

A medium scale (150 ml) transfection was performed in HEK293F cells to test for expression of the huCAN20G2 mAb. A total of $1.5x10^8$ cells were transfected with 150 µg of DNA. The supernatant was harvested by centrifugation (3000 rpm, 15 min, RT) 3 days and 7 days post-transfection. The transfected supernatant was filtered through a 0.22 µm filter. The filtered supernatant from the medium scale transfection was screened with an ELISA prior to purification. An ELISA was run to test the binding of the human mAb clones against whole toxin A and toxin A fragment 4. The human mAb clones were compared to CDA1 and the chimeric CAN20G2. The ELISA plate was coated with 100 µg/ml of Toxin A fragment 4 and 400 µg/ml of whole Toxin A so that the coats were equimolar. The coats were probed with serially diluted mAb (0.128 ng/ml to10 µg/ml) and binding was detected with anti-human IgG-HRP antibody. The plate was read at 405 nm after 60 min incubation with substrate.

Results: As shown in Figures 21 a-d, all three humanized versions of mAb CAN20G2, in addition to the chimeric version, bind to whole toxin A with similar intensity in ELISA. In contrast, there are clearly differences in the binding of the humanized mAbs to recombinant toxin A fragment 4, which is the domain of Tcd A to which the parental CAN20G2 is known to map and bind. This may be indicative of the functionality if this binding to fragment 4 correlates with *in vitro* and *in vivo* protection and may allow the development of domain 4 assays as a surrogate for CAN20G2

efficacy. The chimeric and HE mAbs appear to bind similarly whereas the CDR mAb binds to a lesser degree and the AVA mAb does not appear to bind to the toxin A fragment 4.

Example 15: In vivo challenge with Tcd A

Based on the in vitro data, the CDR and HE humanized versions of CAN20G2 were tested *in vivo* and compared to the chimeric version in the mouse lethal toxin challenge model (as noted in Example 8 above). Swiss Webster mice weighing 20-30g were given 250ug of mAb or controls at day 0 and allowed to rest. After 24 hours, the mice were administered a lethal dose of TcdA (100 ng). This dose kills 100% of animals by 24 hours in an unprotected state. The mice were observed for a period of 4 days for clinical symptoms, abnormality and local and systemic disease. All observations were recorded and the results summarized in Table 13 which shows all the antibodies tested, including the HE and CDR versions are effective at neutralizing toxin A and protecting against toxin A challenge *in vivo*.

Table 13 Effect of Can20G2 humanized MAbs against Tcd A challenge in mice.

Groups	Treatment	N	# Survivors	# Dead/euthanized during the study
A	**chimeric-Can20G2**	5	5	0
B	**HE- Can20G2**	5	5	0
C	**hCDR-Can20G2**	5	5	0
D	**muCan20G2**	5	5	0
E	**CDA1-1**	5	5	0
F	**Rb-polyclonal**	5	5	0
G	**TcdA/PBS controls**	5	0	5
H	**PBS alone**	4	4	0

Example 16: Immunogenicity Analysis of Humanized Antibodies

In order to determine their immunogenicity, CDR-huCAN20G2 and HE-huCAN20G2 were tested in the EpiScreen™ (Antitope Ltd) time course T cell assays, using two markers (proliferation and IL-2 production) to measure T cell activation.

Specifically, peripheral blood mononuclear cells (PBMCs) were prepared from a cohort of 21 healthy donors with representing HLA (Human Leukocyte Antigen) allotypes. Bulk cultures were established using $CD8^+$-depleted PBMCs. $CD4^+$ T cell proliferation by incorporation of [^{3}H]-Thymidine was measured at various time points after the addition of the antibodies. IL-2 secretion was also measured using ELISpot assays in parallel to the proliferation analysis.

Methods

Preparation and selection of donor PBMCs

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy community donor buffy coats (from blood drawn within 24 hours). PBMCs were isolated from buffy coats by Lymphoprep (Axis-shield, Dundee, UK) density centrifugation and $CD8^+$ T cells were depleted using $CD8^+$ RosetteSep™ (StemCell Technologies Inc, London, UK). Donors were characterized by identifying HLA-DR haplotypes using an HLA SSP-PCR based tissue-typing kit (Biotest, Solihull, UK). T cell responses to a control antigen (Keyhole Limpet Haemocyanin (KLH), [Pierce (Perbio), Cramlington, UK]), as well as peptides derived from Influenza A and Epstein Barr viruses were also determined. PBMCs were then frozen and stored in liquid nitrogen until required.

Preparation of Antibodies

The two test antibodies were diluted in AIM-V® culture medium (Invitrogen, Paisley, UK) just before use and the final assay concentration was 0.3mM. KLH was used as a reproducibility control and stored at -20°C as a 10mg/ml stock solution in water. For the studies, an aliquot of KLH was thawed before immediately diluting to 400µg/ml in AIM-V® (final concentration 100µg/ml). Phytohaemagglutanin (PHA, Sigma, Poole, UK) was used as a positive control in the ELISpot and a 1mg/ml stock was stored at -20°C before diluting to a final concentration of 2.5µg/ml in cell cultures.

Assessment of cell viability

On day 7, bulk cultures (previously established for the proliferation assay) were gently resuspended and 10ml of each sample was removed from all donors and mixed

with 10ml trypan blue. These samples were then assessed for viability using trypan blue dye exclusion with a Countess® Automated Cell Counter instrument (Invitrogen).

EpiScreen™ time course T cell proliferation assays

PBMCs from each donor were thawed, counted and viability assessed. Cells were revived in room temperature AIM-V® culture medium, washed and resuspended in AIM-V® to 4-6x10^6 PBMC/ml. For each donor, bulk cultures were established in which 1ml proliferation cell stock was added to the appropriate wells of a 24 well plate. 0.5ml of culture medium and 0.5ml of each diluted antibody were added to the PBMC to give a final concentration of 0.3μM. For each donor, a reproducibility control (cells incubated with 100μg/ml KLH), a positive control (cells incubated with 2.5μg/ml PHA) and a culture medium-only well were also included. Cultures were incubated for a total of 8 days at 37°C with 5% CO_2. On days 5, 6, 7 and 8, the cells in each well were gently resuspended and 3 x 100μl aliquots transferred to each well of a round bottomed 96 well plate. The cultures were pulsed with 0.75μCi [^{3}H]-Thymidine (Perkin ElmerR, Beaconsfield, UK) in 100μl AIM-VR culture medium and incubated for a further 18 hours before harvesting onto filter mats (Perkin ElmerR) using a Skatron Micro 96S - 10056 cell harvester. Counts per minute (cpm) for each well were determined by Meltilex™ (Perkin ElmerR) scintillation counting on a 1450 Microbeta Wallac Trilux Liquid Scintillation Counter (Perkin ElmerR) in paralux, low background counting.

EpiScreen™ IL-2 ELISpot assays

Homologous donors to those used in the proliferation assay were also used for the IL-2 ELISpot assay. Cells were thawed and revived as described above. ELISpot plates (Millipore, Watford, UK) were pre-wetted and coated overnight with 100μl/well IL-2 capture antibody (R&D Systems, Abingdon, UK) in PBS. Plates were then washed 3 times in PBS, incubated overnight in blocking buffer (1% BSA in PBS) and washed in AIM-V® medium. The cell density for each donor was adjusted to 4-6x10^6 PBMC/ml in AIM-V® culture medium and 100μl of cells were added to each well. 50μl of samples and controls were added to the appropriate wells as well as 50ml of AIMV to bring the total volume to 200ml/well. Antibodies were tested in sextuplicate cultures and, for each

donor, a negative control (AIM-V® medium alone), no cells control and a mitogen positive control (PHA at 2.5μg/ml - used as an internal test for ELISpot function and cell viability), were also included on each plate. After an 8 day incubation period, ELISpot plates were developed by sequential washing in dH_2O and PBS (x3) prior to the addition of 100μl filtered, biotinylated detection antibody (R&D Systems) in PBS / 1% BSA. Following incubation at 37°C for 1.5 hours, plates were further washed in PBS (x3) and 100μl filtered streptavidin-AP (R&D Systems) in PBS /1% BSA was added for 1.5 hours (incubation at room temperature). Streptavidin-AP was discarded and plates were washed in PBS (x4). 100μl BCIP/NBT substrate (R&D Systems) was added to each well and incubated for 30 minutes at room temperature. Spot development was stopped by washing the wells and the backs of the wells three times with dH_2O. Dried plates were scanned on an Immunoscan® Analyser and spots per well (spw) were determined using ImmunoscanR Version 4 software.

EpiScreen™ data analysis

For proliferation and IL-2 ELISpot assays, an empirical threshold of a stimulation index (SI) equal to or greater than 2 (SI≥2.00) has been previously established, whereby samples inducing responses above this threshold are deemed positive (borderline SIs ≥ 1.90 are also highlighted). Extensive assay development and previous studies have shown that this is the minimum signal-to-noise threshold allowing maximum sensitivity without detecting large numbers of false positive responses or omitting subtle immunogenic events. For both proliferation (n=3) and IL-2 ELISpot data (n=6) sets, positive responses were defined by statistical and empirical thresholds as follows:

1. Significance ($p<0.05$) of the response by comparing cpm or spw of test wells against medium control wells using unpaired two sample student's t-test.
2. Stimulation index greater than or equal to 2 (SI≥2.00), where SI = mean of test wells (cpm or spw) / baseline (cpm or spw). Data presented in this way is indicated as SI≥2.00, $p<0.05$.

In addition, intra-assay variation was assessed by calculating the coefficient of variance and standard deviation (SD) of the raw data from replicate cultures.

Results & Discussion

While there is generally a good correlation between IL-2 production and proliferation after T cells have been activated, proliferation and IL-2 ELISpot assays have been interpreted independently. Inter-assay variability was assessed using KLH as a reproducibility control where the frequency of positive T cell responses against KLH were compared in two separate EpiScreen™ assays. The results show that interassay variability for KLH-specific T cell responses is within the acceptable range and consistent with previous studies (≤10 %).

Assessment of cell viability

An initial assessment of any gross effect of the antibodies and the buffer on PBMC viability was performed for 10 donors used in the EpiScreen™ time course assays. Cell viabilities were calculated using trypan blue dye exclusion of PBMC 7 days after culture with the antibodies. It was clear that the two test antibodies and buffer formulation did not significantly affect the viability of the cells because PBMC from medium alone cultures had a mean viability similar to that of the samples and KLH treated cells (between 93-97%).

EpiScreen™ time course proliferation assay

Figure 24 and Table 12 show the results obtained in the EpiScreen™ time course T cell proliferation assay of CD4+ T cell responses induced by the antibodies. Both test antibodies induced positive proliferation responses with SI≥2.00 (p<0.05) in one or more donors in the proliferation assay. Borderline responses SI≥1.90 (p<0.05) are also highlighted. Positive proliferation responses ranged between 5% and 24% of the donor cohort (Table 14).

Table 14 Summary of T cell proliferation and IL-2 ELISpot responses

Donor	CDR-hu CAN20G2	HE-hu CAN20G2	Buffer	KLH
Donor 1				PE
Donor 2				PE
Donor 3	**PE**	**PE**		PE
Donor 4	**PE***			PE
Donor 5				PE
Donor 6				PE
Donor 7	**PE‡**			E
Donor 8				PE
Donor 9				PE
Donor 10				PE
Donor 11			N/A	PE
Donor 12			N/A	PE
Donor 13			N/A	PE
Donor 14	**PE**		N/A	E
Donor 15			N/A	PE
Donor 16	**PE**		N/A	E
Donor 17			N/A	PE
Donor 18			N/A	P
Donor 19			N/A	PE
Donor 20			N/A	PE
Donor 21			N/A	PE
Proliferation %	**24**	**5**	**0**	**86**
ELISpot %	**24**	**5**	**0**	**95**
Proliferation and ELISpot %	**24**	**5**	**0**	**81**
Correlation %	**100**	**100**	**N/A**	**94**

In Table 14, during the entire time course (days 5-8), positive T cell proliferation responses (SI≥2.00, significant $p<0.05$) were indicated as "P", and positive T cell IL-2 ELISpot responses (SI≥2.00, significant $p<0.05$) were indicated as "E". Borderline responses (significant $p<0.05$ with SI≥1.90) was shown as (*).No data was obtained on day 8 of the proliferation assay for donor 7 (‡). Formulation buffer was tested on donors 1-10 only donor 11-21 were not tested with the buffer (grey boxes). N/A indicated no data is available.

Antibody CDR-HuCAN20G2 was associated with the most frequent T cell proliferation response, inducing positive responses in 24% (5 donors) of the study cohort. In contrast, antibody HE-HuCAN20G2 induced fewer T cell proliferation responses with only 5% of the cohort responding positively. These results showed that the frequency of T cell proliferation responses is high for antibody CDR-HuCAN20G2 but low for HE-HuCAN20G2. No T cell proliferation responses were detected against the buffer control.

Analysis of the magnitude of T cell proliferation responses showed that although antibody CDR-HuCAN20G2 had a high frequency of response, the magnitude of responses were low (mean SI 2.13). For antibody HE-HuCAN20G2 no conclusions can be made regarding the magnitude of the T cell response due to the low number of responding donors (**Table 15**). Thus, the overall immunogenic potential of the antibodies was determined based on the frequency (%) of the positive T cell proliferation responses in the study cohort with CDR-HuCAN20G2 being more immunogenic than HE-HuCAN20G2.

Table 15 Summary of the mean magnitude (±SD) of positive T cell proliferation responses against the antibodies.

Sample	**Mean SI**	**+/- SD**	**Frequency (%) of Response**
CDR-HuCAN20G2	2.13	0.09	24
HE-HuCAN20G2	2.25	0.13	5
KLH	2.60	0.78	86

The mean SI was calculated from the average of all positive donor responses observed during the entire time course (days 5-8). The data includes borderline proliferation responses (SI≥1.90, $p<0.05$).

Kinetics of T cell responses

The overall timing of the proliferative responses can provide information as to the potential type of T cell response (naïve or recall). Maximal T cell proliferation detected on day 5 indicates that existing T cell precursor frequencies are high, whereas maximal proliferation on later days indicates a low existing T cell precursor frequency. A high immunogenic potential would be concordant with stimulation of T cells during the early phase of the time course. Figure 25 summarizes the number of positive proliferation responses occurring against the samples on each day of the four day time course. The T cell responses against antibody CDR-HuCAN20G2 were observed mostly on days 7 and 8, suggesting that for this antibody the number of existing T cell precursors is low. Antibody HE-HuCAN20G2 induced one donor to respond and this was observed on days 6, 7 and 8. However, since only one responding donor was detected it is difficult to make a conclusion as to the number of T cell precursors for antibody HE-HUCAN20G2.

EpiScreen™ IL-2 ELISpot assay

Figure 26 and Table 12 show the responses obtained in the IL-2 ELISpot assay which measures IL-2 secretion by CD4+ T cells following stimulation with the two test antibodies. Similar to the proliferation assay, positive responses were recorded in donors that produced an SI≥2.00 with a significant ($p<0.05$) difference observed between test spw and background (untreated medium control). Borderline responses SI≥1.90 ($p<0.05$) are also highlighted. All samples induced positive IL-2 ELISpot responses in one or more donors and these were all significant ($p<0.05$) using an unpaired, two sample student's t-test. All PHA wells were positive for the presence of spots although SI values were not prepared for the ELISpot data as, after 8 days, the majority of wells contained spots too numerous to count (data not shown).

For the two test antibodies, the overall results of the IL-2 ELISpot assay were homologous to those obtained in the proliferation assay with both antibodies inducing the

same frequency of T cell responses (Table 16). As in the proliferation assay, antibody CDR-HuCAN20G2 induced the most frequent T cell responses in the study cohort with 24% of donors responding positively (SI≥2.00, $p<0.05$), whereas antibody HE-HuCAN20G2 induced T cell responses in 5% of the study cohort. Assessment of the mean magnitude of positive (including borderline SI≥1.90, $p<0.05$) T cell responses against both antibodies was low (mean positive SI 2.39 for CDR-HuCAN20G2).

The frequency of T cell responses was low for HE-HuCAN20G2 which precludes making any direct correlation between strength of T cell response (magnitude) and immunogenicity. Assessment of the relative risk of immunogenicity of the test antibodies (based on the frequency of positive responses in the IL-2 ELISpot assay) showed that CDR-HuCAN20G2 was more immunogenic than HE-HuCAN20G2.

Table 16 Summary of the mean magnitude (±SD) of positive T cell IL-2 secretion responses against the antibodies.

Sample	Mean SI	+/- SD	Frequency (%) of Response
CDR-HuCAN20G2	2.39	0.52	24
HE-HuCAN20G2	2.63	N/A	5
KLH	4.13	1.48	95

The data includes borderline responses (SI≥1.90, $p<0.05$). N/A indicates no data available.

Interpretation of results

The proliferation and IL-2 ELISpot assay data show that positive T cell responses were detected against both test antibodies in a proportion of the donors. The overall correlation between proliferation and IL-2 ELISpot assays was high (94% for KLH, Table 14) and thus, as in previous studies, responding donors were defined as those that mounted a positive response to each sample in both IL-2 ELISpot and proliferation assays. Table 14 shows a summary of positive responses against the antibodies in both proliferation and IL-2 ELISpot assays. Comparison of the data obtained from the

proliferation and IL-2 ELISpot assays showed that the antibodies tested induced homologous frequencies of positive T cell responses between the assays. All donors produced a positive T cell response against PHA in the IL-2 ELISpot assay indicating that cells in the *ex vivo* cultures were functional (data not shown). Analysis of the combined datasets from these two assays revealed that the overall frequency and magnitude of responses was high for antibody CDR-HuCAN20G2 with 24% of donors responding in both proliferation and ELISpot assays and low for antibody HE-HuCAN20G2 with 5% of donors responding.

Conclusion

The overall correlation between proliferation and IL-2 ELISpot assay was high, responding donors were defined as those that mounted a positive response to each sample in both assays. Analysis of the combined datasets from two assays reveals that overall response was high for antibody CDR-huCAN20G2 with 24% of donors responding in both assays and low for antibody HE-huCAN20G2 with 5% of donors responding. Previous EpiScreen™ T cell assays with a range of biologics have showed a clear correlation between the percentage of donor T cell responses in the assay and the level of immunogenicity observed in clinic, whereas the protein therapeutics that induced >10% positive response are associated with risk of immunogenicity in the clinic. The current study results showed that, in comparison with other protein therapeutics tested in EpiScreen™ assays, antibody CDR-huCAN20G2 would be considered as having a risk of clinical immunogenicity. In contrast, antibody HE-huCAN20G2 would be considered as having a low risk of clinical immunogenicity.

Example 17: In vivo efficacy of humanized CAN20G2 mAbs against toxin A challenge

The *in vivo* protective efficacy of the two humanized CAN20G2 anti-TcdA mAbs, HE-CAN20G2 and CDR-CAN20G2 were evaluated in the mouse lethal toxin challenge model (as noted in Example 8 above) by testing a low dose of antibody. Swiss Webster mice weighing 20-30g were given 50ug of mAb or controls at day 0 and allowed to rest. After 24 hrs, the mice were given a lethal dose of TcdA (100 ng). This dose kills 90-

100% of animals by 24 hours in an unprotected state. The mice were observed for a period of 14 days for clinical symptoms, abnormality and local and systemic disease. All observations were recorded and the % survival was determined for each treatment group.

Results

As shown in Figures 27 and 28, both humanized CAN20G2 mAbs were efficacious in protecting against toxin A *in vivo* challenge. HE-CAN20G2 conferred better *in vivo* protection compared to CDA1 and CDR-CAN20G2. At the low dose of 0.05mg/mouse, HE-CAN20G2 recipient mice had a higher survival rate (90%) compared to those treated with CDA1 (80%) and CDR-CAN20G2 (70%) against TcdA lethal challenge.

Example 18: Pharmacokinetic Analysis of Humanized Antibodies

Pharmacokinetic studies were conducted for CDR-huCAN20G2 and HE-huCAN20G2 in hamster model and rat model. In hamster study, Golden Syrian hamsters were injected intraperitoneally with 50 mg/kg of CDR-huCAN20G2. Blood samples were collected at 2h, 24h, 48h, 72h, 96h, 168h, 240h and 336h post-injection. Control samples were collected from test animal 5 days before injection and sentinel group at different time points. The blood samples were centrifuged at 8000 rpm for 10 minutes to obtain sera. In rat study, two groups of Sprague-Dawley rats were instrumented with a femoral vein catheter (FVC) for intravenous dosing and a jugular vein catheter (JVC) for blood collection. Two antibodies, CDR-huCAN20G2 and HE-huCAN20G2, were injected to each group of rats at 10 mg/kg dose level via single IV bolus followed by 0.5 mL saline flush. Blood samples were collected at pre-dose, 0.083, 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288 and 312 hours post-dose from the JVC. Whole blood (300 μL) samples were centrifuged at 2200 x g for 10 minutes to isolate sera.

The antibody concentration in the sera was determined via ELISA. 96-well ELISA plate were coated overnight with goat anti-human IgG, affinity purified and monkey serum adsorbed (Novus Biologicals) at 1μg/mL. Plates were washed with PBS-T and blocked with blocking buffer. The antibody reference standard was diluted in 1% pooled naïve hamster serum to generate a standard curve with a range of 0.098 – 100

ng/mL. Diluted test samples and standards were incubated 1.5 hours at room temperature. Plates were washed and incubated with HRP-goat anti-human IgG, affinity purified and monkey serum adsorbed (Novus Biologicals), developed with TMB peroxidase substrate system (R&D systems) and stopped with TMB peroxidase stop solution (R&D system). Plates were read on a SpectraMax plate reader at 450 nm. Antibody concentration in each animal at different time points as calculated using the standard curves.

Results: For hamster PK study, noncompartmental pharmacokinetic analysis was performed using SAS Version 9.2 for Windows, the data are shown in Table 17. As indicated, CDR-huCAN20G2 demonstrated a terminal half life around 6 days with 50 mg/kg administration dose, which ensured antibody retention in future efficacy studies.

For rat PK study, noncompartmental pharmacokinetic analysis was performed using Watson, version 7.2.0.02 and the data are illustrated in Table 18 and Figures 29A and 29B. As indicated, the PK profiles of the two monoclonal antibodies are very similar. Comparable levels of exposure were exhibited and metabolism was close to the same rate.

Table 17 PK Study of Humanized Antibodies in Hamsters

mAb	Cmax (µg/mL)	Tmax (hour)	$AUC_{(0-t)}$ (µg*hour/mL)	t½ Half-life (hour)
CDR-huCAN20G2 50 mg/kg	244.9	24	36777.5	166.44

Table 18 PK Study of Humanized Antibodies in Rats

mAb	$AUC_{(0-x)}$ µg*hour/mL	$Cl_{(0-x)}$ mL/kg/hr	$Vd_{ss(0-x)}$ mL/kg	t½ Half-life (hour)
CDR-huCAN20G2 10mg/kg	14533	0.689	81.8	170
HE-huCAN20G2 10mg/kg	17500	0.573	70.2	209

While specific aspects of the invention have been described and illustrated, such aspects should be considered illustrative of the invention only and not as limiting the invention as construed in accordance with the accompanying claims. All publications and patent applications cited in this specification are herein incorporated by reference in their entirety for all purposes as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference for all purposes. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

1. An isolated antibody or antigen-binding portion thereof comprising a heavy chain variable region and a light chain variable region,

wherein the heavy chain variable region comprises three complementarity determining regions (CDRs), CDR1, CDR2, and CDR3, having the amino acid sequences set forth in SEQ ID NOs: 29, 30, and 31, respectively;

wherein the light chain variable region comprises three CDRs, CDR1, CDR2, and CDR3, having the amino acid sequences set forth in SEQ ID NOs: 21, 22, and 23, respectively; and

wherein the antibody or antigen-binding portion thereof specifically binds to *Clostridium difficile* (*C. difficile*) toxin A.

2. An isolated antibody heavy chain variable region that comprises three CDRs, CDRl, CDR2 and CDR3, having the amino acid sequences set forth in SEQ ID NOs: 29, 30 and 31, respectively,

wherein an antibody or antigen-binding portion thereof comprising the heavy chain variable region and a light chain variable region comprising the amino acid sequence SEQ ID NO: 20 can specifically bind to *C. difficile* toxin A.

3. An isolated antibody light chain variable region that comprises three CDRs, CDRl, CDR2 and CDR3, having the amino acid sequences set forth in SEQ ID NOs: 21, 22 and 23, respectively,

wherein an antibody or antigen-binding portion thereof comprising the light chain variable region and a heavy chain variable region comprising the amino acid sequence SEQ ID NO: 28 can specifically bind to *C. difficile* toxin A.

4. The antibody or antigen-binding portion thereof of claim 1 or 2, wherein the heavy chain variable region comprises the amino acid sequence SEQ ID NO: 28.

5. The antibody or antigen-binding portion thereof of claim 1 or 3, wherein the light chain variable region comprises the amino acid sequence SEQ ID NO: 20.

6. The antibody or antigen-binding portion thereof of any one of claims 1 to 3, wherein the heavy chain variable region comprises the amino acid sequence SEQ ID NO: 28 and the light chain variable region comprises the amino acid sequence SEQ ID NO: 20.

7. The antibody or antigen-binding portion thereof of claim 1, wherein the heavy chain variable region comprises the amino acid sequence SEQ ID NO: 89 and wherein the light chain variable region comprises the amino acid sequence SEQ ID NO: 91.

8. The antibody or antigen-binding portion thereof of claim 1, wherein the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 93 and wherein the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 95.

9. The antibody or antigen-binding portion thereof of any one of claims 1 to 8, wherein the antibody or antigen-binding portion thereof is humanized or chimeric.

10. The antibody or antigen-binding portion thereof of any one of claims 1 to 9, wherein the antigen-binding portion thereof is a whole immunoglobulin molecule, an scFv, a Fab fragment, an F(ab′)2, or a disulfide-linked Fv.

11. The antibody or antigen-binding portion thereof of any one of claims 1 to 10, wherein the antibody or antigen-binding portion thereof comprises at least one constant domain selected from the group consisting of an IgG constant domain and an IgA constant domain.

12. The antibody or antigen-binding portion thereof of any one of claim claims 1 to 11, wherein the antibody or antigen-binding portion thereof binds to fragment 4 of *C. difficile* toxin A.

13. The antibody or antigen-binding portion thereof of any one of claims 1 to 12, wherein the antibody or antigen-binding portion thereof is functionally linked to another molecule.

14. The antibody or antigen-binding portion thereof of claim 13, wherein the molecule is an antibody, detectable agent, a cytotoxic agent, a pharmaceutical agent, a protein or peptide that mediates association with another molecule, an amino acid linker, a signal sequence, or an immunogenic carrier.

15. A composition comprising the antibody or antigen-binding portion thereof of any one of claims 1 to 14, and at least one pharmaceutically acceptable carrier.

16. A kit comprising the antibody or antigen-binding portion thereof of any one of claims 1 to 14.

17. The kit of claim 16, further comprising: instructions for use; a therapeutic agent; a coupling agent; one or more of *C. difficile* whole toxin A, toxoid A, toxin A fragment 4, whole toxin B, toxoid B, toxin B fragment 4; or a pharmaceutically acceptable carrier.

18. Use of an effective amount of the antibody or antigen-binding portion thereof of any one of claims 1 to 14 in the manufacture of a medicament for preventing or treating *C. difficile*-associated disease.

19. The use of claim 18, wherein a dosage of the medicament comprises an effective amount of the antibody or antigen binding portion thereof.

20. The use of claim 18 or 19, wherein the antibody or antigen-binding portion thereof is to be administered intravenously, subcutaneously, intramuscularly or transdermally.

21. The use of any one of claims 18 to 20, wherein the medicament further comprises a second agent.

22. The use of claim 21, wherein the second agent is a different antibody or antigen-binding portion thereof.

23. The use of claim 21, wherein the second agent is an antibiotic.

24. The use of claim 23, wherein the antibiotic is vancomycin, metronidazole, or fidaxomicin.

25. A method of detecting *Clostridium difficile* (*C. difficile*) toxin A, comprising the steps of contacting a sample suspected or known to contain *C. difficile* toxin A with the antibody or antigen-binding portion thereof of any one of claims 1 to 14 and detecting binding of the antibody or antigen-binding portion thereof to *C. difficile* toxin A.

26. A method of isolating *Clostridium difficile* (*C. difficile*) toxin A from a sample, which comprises contacting a sample comprising or suspected of comprising *C. difficile* toxin A with the antibody or antigen-binding portion thereof of any one of claims 1 to 14.

27. An isolated polynucleotide comprising a nucleic acid that encodes the heavy chain variable region or the light chain variable region of an antibody or antigen-binding portion thereof of any one of claims 1 to 12.

28. The polynucleotide of claim 27, comprising the nucleic acid sequence SEQ ID NO: 70.

29. The polynucleotide of claim 27, comprising the nucleic acid sequence SEQ ID NO: 71.

30. The polynucleotide of claim 27, comprising the nucleic acid sequence SEQ ID NO: 88.

31. The polynucleotide of claim 27, comprising the nucleic acid sequence SEQ ID NO: 90.

32. The polynucleotide of claim 27, comprising the nucleic acid sequence SEQ ID NO: 92.

33. The polynucleotide of claim 27, comprising the nucleic acid sequence SEQ ID NO: 94.