



A monoclonal antibody collection for *C. difficile* typing?

Lise Hunault, Patrick England, Frédéric Barbut, Bruno Iannascoli, Ophélie Godon, François Déjardin, Christophe Thomas, Bruno Dupuy, Chunguang Guo, Lynn Macdonald, et al.

► To cite this version:

Lise Hunault, Patrick England, Frédéric Barbut, Bruno Iannascoli, Ophélie Godon, et al.. A monoclonal antibody collection for *C. difficile* typing?. *Gut Pathogens*, 2024, 16 (1), pp.4. 10.1186/s13099-023-00592-7 . hal-04441824v2

HAL Id: hal-04441824

<https://hal.sorbonne-universite.fr/hal-04441824v2>

Submitted on 6 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

BRIEF REPORT

Open Access



A monoclonal antibody collection for *C. difficile* typing ?

Lise Hunault^{1,2,3}, Patrick England⁴, Frédéric Barbut^{5,6}, Bruno Iannascoli¹, Ophélie Godon¹, François Déjardin⁷, Christophe Thomas⁷, Bruno Dupuy⁸, Chunguang Guo⁹, Lynn Macdonald⁹, Guy Gorochov^{2*†}, Delphine Sterlin^{2†} and Pierre Bruhns^{1*†}

Abstract

Clostridioides difficile is the leading cause of antibiotic-associated diarrhea and pseudomembranous colitis in adults. Various *C. difficile* strains circulate currently, associated with different outcomes and antibiotic resistance profiles. However, most studies still focus on the reference strain 630 that does not circulate anymore, partly due to the lack of immunological tools to study current clinically important *C. difficile* PCR ribotypes. The goal of this study was to generate monoclonal antibodies recognizing various epidemic ribotypes of *C. difficile*. To do so, we immunized mice expressing human variable antibody genes with the Low Molecular Weight (LMW) subunit of the surface layer protein SlpA from various *C. difficile* strains. Monoclonal antibodies purified from hybridomas bound LMW with high-affinity and whole bacteria from current *C. difficile* ribotypes with different cross-specificities. This first collection of anti-*C. difficile* mAbs represent valuable tools for basic and clinical research.

Keywords *Clostridioides difficile*, Monoclonal antibodies, S-layer, Hybridomas, Ribotypes

Introduction

Clostridioides difficile is an anaerobic, gram-positive, and spore-forming bacterium that is the main agent responsible for antibiotic-associated diarrhea and pseudomembranous colitis in adults [1]. In the past decades, there was a drastic increase in the incidence of both healthcare-associated *C. difficile* infection (CDI) and community-acquired CDI [2]. There is a large phylogenetic diversity of *C. difficile* with more than 300 distinct PCR-ribotypes (RT) reported worldwide, including epidemic lineages associated with increased transmission and mortality [3–6]. The latest epidemiology data worldwide reported that 5 ribotypes i.e., RT001, RT002, RT014, RT027 and RT078, account for approximately 50% of the infections [7].

Whereas several advances such as fluorescent mutants and novel fingerprinting techniques have contributed to a better understanding of *C. difficile* diversity and physiology [8–10], basic research still relies on one single strain i.e., *C. difficile* 630 that belong to RT012. An

†Guy Gorochov, Delphine Sterlin and Pierre Bruhns shared senior authorship.

*Correspondence:

Guy Gorochov

guy.gorochov@sorbonne-universite.fr

Pierre Bruhns

pierre.bruhns@pasteur.fr

¹ Antibodies in Therapy and Pathology, Institut Pasteur, Université Paris Cité, INSERM UMR1222, 75015 Paris, France

² Centre d'Immunologie et des Maladies Infectieuses (CIMI-Paris), Sorbonne Université, INSERM, CNRS, 75013 Paris, France

³ Sorbonne Université, Collège doctoral, 75005 Paris, France

⁴ Plateforme de Biophysique Moléculaire, Institut Pasteur, Université Paris Cité, CNRS UMR3528, 75015 Paris, France

⁵ National Reference Laboratory for *Clostridium difficile*, 75012 Paris, France

⁶ Université Paris Cité, INSERM UMR-1139, Paris, France

⁷ Production and Purification of Recombinant Proteins Facility, Institut Pasteur, 75015 Paris, France

⁸ UMR-CNRS 6047, Laboratoire Pathogenèse des Bactéries Anaérobies, Institut Pasteur, Université Paris-Cité, 75015 Paris, France

⁹ Regneron Pharmaceuticals, Tarrytown, NY, USA



increasing number of studies has been performed on the epidemic ribotype 027, which caused major outbreaks in the United States and Europe at the end of the 2010s [11, 12]. Other ribotypes remain largely unexplored even though some are associated with antibiotic resistance and increased severity [3], which can be partly explained by the lack of genetic and immunological tools to study these strains.

C. difficile surface is composed of adhesins e.g., the flagellar cap protein FliD, the flagellin FliC, the cell wall protein Cwp66, the surface layer protein SlpA, and the protease Cwp84 [13]. SlpA is expressed on the bacterial surface of all ribotypes and plays a crucial role in the pathogenesis and virulence of *C. difficile* by mediating interactions with the host cells and the surrounding environment [14–17]. SlpA contains two biologically distinct entities, the high-molecular weight (HMW) and the low molecular weight (LMW) subunits that assemble on the bacterial surface into a paracrystalline lattice [18]. Sequence variations of SlpA have been reported for the LMW that correlate with the diversity of clinical isolates, whereas the HMW is less variable [19, 20]. SlpA is highly immunogenic, meaning it can trigger an immune response in the host [21]. Indeed, antibodies against SlpA have been detected in the sera of patients infected with *C. difficile*, indicating its potential as a target for vaccine development [21, 22].

In this work, we generated the first collection of mAbs that bind and discriminate predominant clinical ribotypes of *C. difficile*. Knock-in mice expressing human antibody variable genes for the heavy (V_H) and light chain (V_L) [23, 24] were immunized with a collection of recombinantly expressed LMW from five clinically relevant *C. difficile* ribotypes i.e., RT001, RT002, RT014, RT027 and RT078. Hybridomas were generated and their corresponding IgG mAbs bound both recombinant LMW in vitro and LMW naturally expressed on the bacterial surface. At least one mAb was identified against each of the five ribotypes used for immunization, with 6 mAbs being cross-reactive between LMW subunits of two different *C. difficile* ribotypes. The reduced sequence identity of LMW between different *C. difficile* ribotypes [25] allows for specific identification of bacterial ribotypes by this anti-LMW mAb collection that represents a novel toolkit for *C. difficile* research.

Results

LMW SlpA subunits from 5 predominant ribotypes of *C. difficile* i.e., RT001, RT002, RT014, RT078 and RT027 (Fig. 1a), were recombinantly produced from transformed *Escherichia coli* as his-tagged soluble proteins and affinity-purified. As anti-LMW antibodies may potentially be of therapeutic interest for the treatment of CDIs, we used

knock-in mice in which the endogenous genes encoding the heavy chain variable domain (VH) and the kappa light chain variable domain (Vκ) were replaced by their human counterparts (Velocimmune mice) [23, 24] with one modification, i.e., only one allele of the endogenous Vκ locus was replaced by human Vκ segments, and the second allele of the endogenous Vκ locus was replaced by human Vλ segments (Fig. 1b). As the Vκ locus expresses 95% of the light chains in mice [26], placing human Vλ segments at the Vκ locus increases the variability of light chain expression. Thus, after hybridoma identification, cloning of these VH and VL into vectors containing human heavy and light chain constant domains, allows for direct development—in fine—of fully human anti-LMW mAbs. To generate hybridomas, mice were immunized at D0, D21 and D42 with 50 µg/mouse of each LMW (Fig. 1c). High anti-LMW IgG serum titers were obtained in all mice at day 42 (Fig. 1d). Mice were boosted with all five LMW at equimolar ratio (Fig. 1c), and their spleen harvested 4 days later. Two different protocols were tested and gave similar results; one based on the similarity between the LMW—grouping two highly similar LMW in a single immunization; one based on their frequency in current CDI—grouping LMW corresponding to current clinical ribotypes in a single immunization (Additional file 1: Fig. 1). More than 700 hybridomas were generated and among them 100 hybridoma were found to secrete anti-LMW antibodies.

Among these 100 hybridomas, the 14 clones displaying the highest ratio of LMW binding by ELISA compared to IgG concentration in their culture supernatant were expanded and their antibodies purified. Their binding profiles towards the five recombinant LMW proteins were assessed by ELISA (Fig. 2). 12 out of 14 (86%) significantly bound LMW-RT001 with variable profiles, 1 out of 14 (7%) bound LMW-RT002, 1 out of 14 (7%) bound LMW-RT014, 6 out of 14 (43%) bound LMW-RT078 and 11 out of 14 (78%) bound LMW-RT027. Among the eleven LMW-RT027-binding mAbs, four (36%) cross-reacted strongly with LMW-RT001 (mAb SG8, TF1, TH4 and VA10) and one with both LMW-RT001 and LMW-RT078 (mAb RF12). mAb QE2 cross-reacted with four LMWs: LMW-RT001, LMW-RT014, LMW-RT027 and LMW-RT078. Among the three mAbs that did not recognize LMW-RT027, mAb RA11 was specific for LMW-RT078, mAb UA5 cross-reacted with LMW-RT001 and LMW-RT002, and mAb SC6 cross-reacted with LMW-RT001 and LMW-RT078.

We next evaluated the affinity of the mAbs displaying the strongest interactions with their respective targets i.e., LMW-RT001, LMW-RT002, LMW-RT014, LMW-RT078 and LMW-RT027, by Bio Layer Interferometry (BLI), coupling IgGs to the sensors and keeping LMW

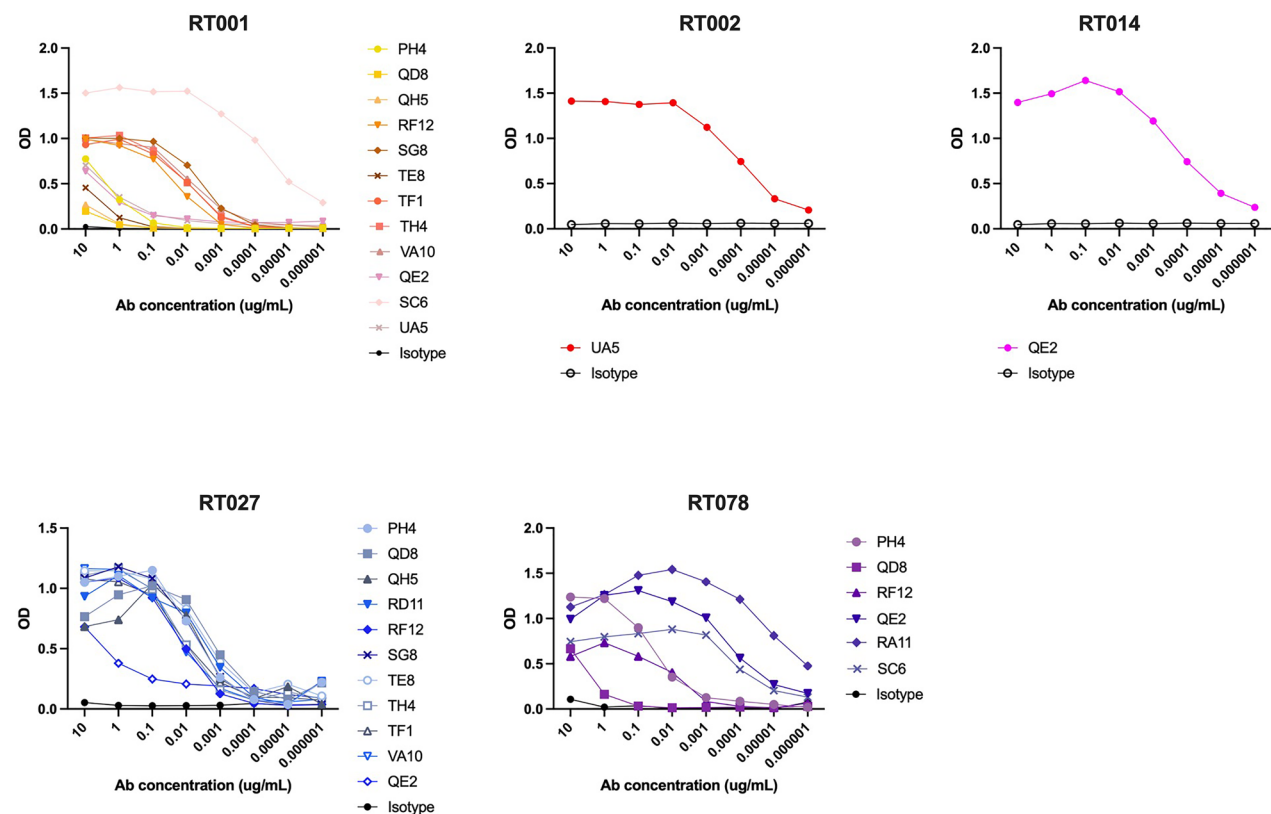


Fig. 2 Specificities of anti-LMW mAbs. ELISA results (OD values 492 nm versus 620 nm) against recombinant LMW-RT001, LMW-RT002, LMW-RT014, LMW-RT078 and LMW-RT027 of IgG mAbs at indicated concentrations. Black curves represent isotype controls

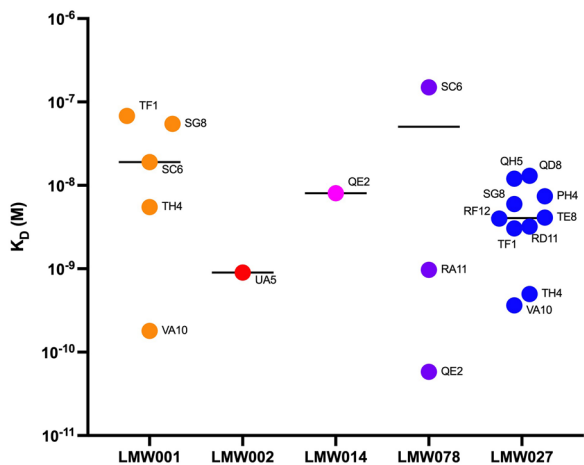


Fig. 3 Affinities of mAbs for the LMW of five clinical ribotypes. Dissociation constant (K_D) values measured by BLI. Each dot represents the K_D value of one mAb (mAb name indicated) interacting with one LMW among LMW-RT001, LMW-RT002, LMW-RT014, LMW-RT078 and LMW-RT027. Black bars represent median K_D values of the group of mAbs binding one ribotype

of these mAbs to the LMW recombinant proteins and the LMW exposed at the bacterial surface for the five clinical ribotypes RT001, RT002, RT014, RT078, RT027.

Finally, we studied the impact of LMW binding by the anti-LMW-RT027 mAbs in an in vitro growth assay on *C. difficile* strain 027. Two monospecific mAbs for LMW-RT027 (QD8 and QH5) and two cross-specific mAbs (VA10 and TH4) were tested for their impact on growth. Growth was followed over 24 h with an isotype control IgG and showed an exponential phase followed by a plateau (Fig. 4b). Anti-LMW-RT027 did not significantly alter growth, even though mAb VA10 tended to delay growth, and mAb QD8 and, to a lesser extent, mAb QH5, tended to increase growth.

Discussion

Herein, we report the first monoclonal antibody collection that targets a surface protein of *C. difficile*. Due to sequence variability in the low-molecular weight subunit of surface layer protein A, this mAb collection allows the detection of 5 different ribotypes of clinical interest.

flow cytometry. Table 1 summarizes the binding profiles

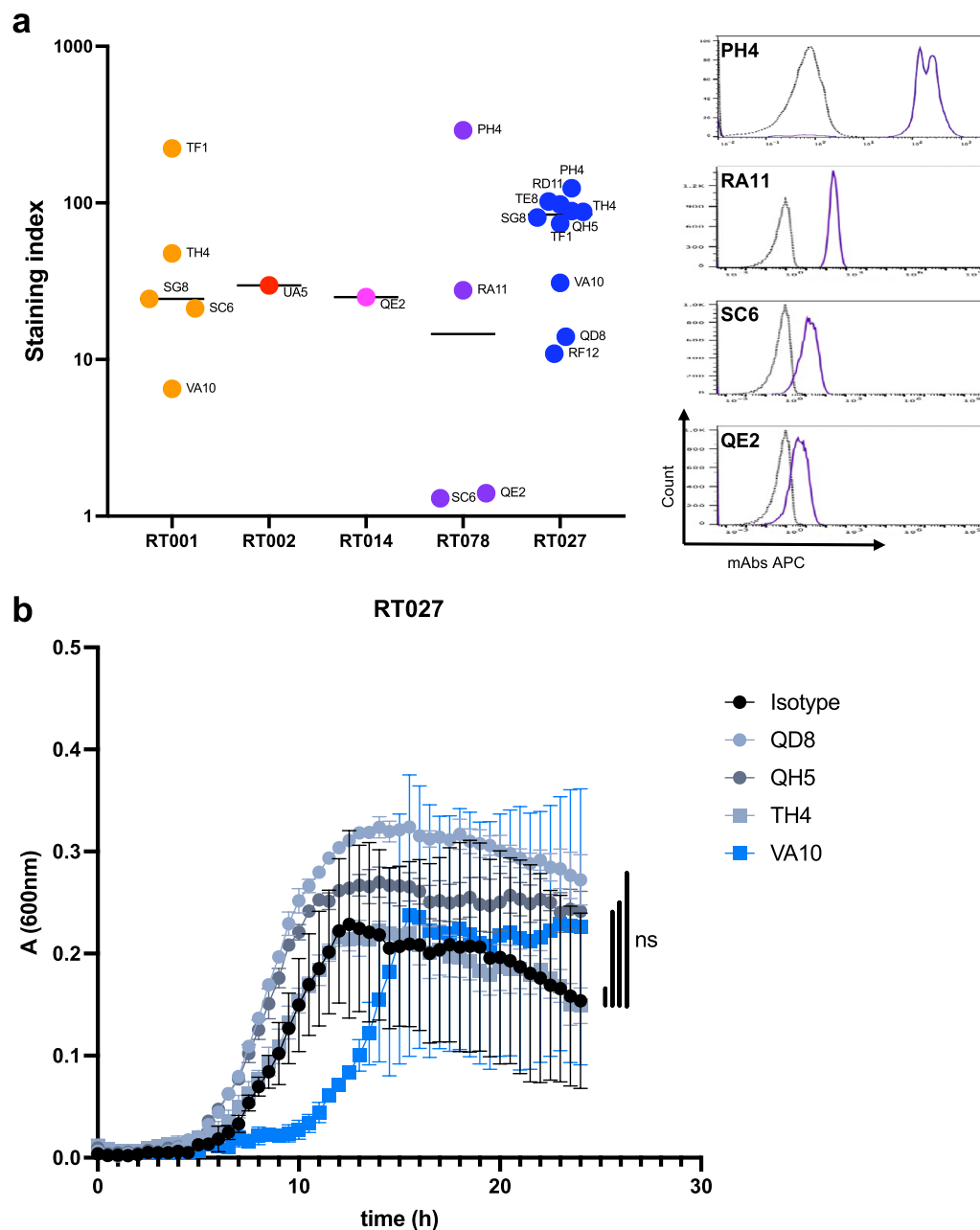


Fig. 4 Binding of mAbs to LMWs expressed at the surface of *C. difficile* bacteria. **a** Right: Flow cytometry analysis of mAbs binding to LMW of indicated *C. difficile* ribotypes. Results are displayed as staining index (refer to methods section). Left: representative histograms for staining of strain RT078 by mAbs PH4, RA11, SC6 and QE2 are shown. **b** Growth of *C. difficile* strain RT027 in BHISG medium incubated with indicated anti-LMW027 mAb or with an unspecific IgG (isotype). Growth was followed continuously over 24 h. Each dot represents the mean of three technical replicates, and the bars indicate standard deviations. ns: non-significant

More than half the mAbs bound selectively to the bacterial surface of one of these ribotypes, whereas the cross-reactive mAbs bound to two different ribotypes. The relatively high affinity of the interaction (nanomolar range) allows to envision using these mAbs for various

assays such as ELISA, flow cytometry, microscopy, or histology assays.

In this study we chose to immunize mice with the low-molecular weight subunit of surface layer protein A as it represents a major antigen of the *C. difficile* surface [27]. Although we found by alignment stretches of conserved

Table 1 Summary table of mAbs binding profiles to LMW recombinant proteins and LMW expressed at the bacterial surface of *C. difficile* bacteria for five clinical ribotypes

Antibody	RT				
	001	002	014	078	027
PH4	E			E/F	E/F
QD8	E			E	E/F
QH5	E				E/F
RD11					E/F
RF12	E			E	E/F
SG8	E/F				E/F
TE8	E				E/F
TF1	E/F				E/F
TH4	E/F				E/F
VA10	E/F				E/F
QE2	E		E/F	E/F	E
RA11				E/F	
SC6	E/F			E/F	
UA5	E	E/F			

E indicates binding by ELISA and F binding by flow cytometry. Blanks indicate absence of binding

residues between the five ribotype sequences we used [18], we could not identify any antibody cross-binding all five strains. The most cross-reactive anti-LMW mAbs recognized by bacterial flow cytometry only two different ribotypes. This suggests that conserved epitopes between LMW of different strains may not be dominant epitopes in terms of immunogenicity or may be hidden or poorly accessible to antibodies. Indeed, conserved amino acids have been implicated in the interaction between the LMW and the High Molecular Weight subunits which face inward toward the bacterial cell wall [28] and are therefore probably inaccessible to antibodies.

Mice were immunized sequentially with five different LMWs and boosted with a mix of all of them, leading to identification of mAbs to each of them. Varying the order of different LMWs in the immunization scheme did not significantly alter antibody titers for the various LMWs, except for LMW-RT001 when injected with a farther ribotype. Antibodies binding SlpA have also been detected in the sera of patients infected with *C. difficile*, suggesting that, indeed, SlpA or its LMW subunit are immunogenic. Even though the knock-in mice we used produce antibodies with human variable domains [23, 24], thus potentially resembling those found in infected patients, we did not identify antibodies that significantly alter bacterial growth in our in vitro assays. It remains unclear whether such antibodies exist in patients in remission or if other mechanisms are at play. Interestingly, 30% of relapsing *C.*

difficile infections are not due to the initial infecting strain but to a different strain, acquired from an exogenous source [29]. Whether the sequence variability of LMW among *C. difficile* ribotypes is involved in this recurrence and escape from the host immune response remains to be investigated.

This novel series of anti-*C. difficile* mAbs contains three anti-LMW mAbs specifically recognizing epidemic ribotypes RT027, bound by mAb TE8, RT078 bound by mAb RA11, and RT002 bound by mAb U5A. These three ribotypes have been associated with poor outcomes after infection [6, 30, 31]. Beyond *C. difficile* 630, the most studied *C. difficile* ribotype, this set of mAbs could help to study ribotypes RT027, RT078 and RT002 by resorting to various assays (ELISA, flow cytometry, microscopy, histology, blotting). One could even propose targeted treatments, by coupling antibiotics to these mAbs (aka Antibody–Drug Conjugates, ADC) to reduce antibiotic doses.

Our study however has limitations. While it has recently been reported, using whole-genome sequencing, that diversity exists within a given ribotype [32], we only tested five ribotypes of *C. difficile*, each derived from a single clinical isolate. Therefore, more clinical isolates now remain to be tested to determine whether mAb specificity encompasses all known strains in each ribotype. Moreover, we only tested cross-specificity towards a limited panel of ribotypes. It remains to be deciphered if these mAbs cross-react with other *C. difficile* ribotypes or even to other closely related microbial pathogens that we did not include herein and that may prevent using this mAb series to conduct detection or ribotyping in clinical samples.

To our knowledge, these mAbs represent the first collection of antibodies against *C. difficile* surface protein SlpA. These mAbs bind LMW from different clinically relevant strains *i.e.*, LMW-RT001, LMW-RT002, LMW-RT014, LMW-RT027 and LMW-RT078. These mAbs represent interesting probes to better understand *C. difficile* infection, pathogenesis, and epidemiology.

Materials and methods

Bacterial strains and culture conditions

Clinical isolates of *C. difficile* RT001, RT002, RT014, RT027, RT078 were provided by The French National Reference Laboratory for *C. difficile*. Strains were grown anaerobically (5% H2, 5% CO2, 90% N2) in TY medium (30 g/L tryptone, 20 g/L yeast extract). All media were purchased from Sigma-Aldrich.

Mice

Knock-in mice expressing human antibody variable genes for the heavy (V_H) and light chain (V_L) (VelocImmune)

were described previously [23, 24] and provided by Regeneron Pharmaceuticals to be bred at Institut Pasteur. All animal care and experimentation were conducted in compliance with the guidelines. The study, registered under #210111 was approved by the Animal Ethics committee CETEA (Institut Pasteur, Paris, France) and by the French Ministry of Research.

Production of recombinant LMW proteins

Recombinant *C. difficile* LMW-SLPs (LMW-RT001, LMW-RT002, LMW-RT014, LMW-RT078, LMW-RT027, LMW630 [25]) were produced as N-terminal 6xHis-tagged proteins from plasmid pET-28a(+) (Twist-Biosciences, #69864). Plasmids were transformed into *E. coli* strain DE3 and grown in NZY auto-induction lysogeny broth (LB) medium (NZYtech, #MB180). Bacteria were harvested by centrifugation and lysed using Cell Disruptor (Constant System) at 1.3 kbar. Recombinant LMW-SLP proteins from the soluble fraction were purified by affinity chromatography on HisTrap FF crude 1 mL columns (Cytiva life science, #29048631) followed by size exclusion chromatography on HiLoad 16/600 Superdex 75 pg (Cytiva life science, #28989333) using an AKTA pure (Cytiva life science). All proteins were stored in 50 mM sodium phosphate buffer pH 7.8, 300 mM NaCl prior to analysis or long-term storage.

Production of LMW-specific monoclonal antibodies

VelocImmune mice were injected i.p. at day 0, 21 and 42 with 50 µg of each of five recombinant LMWs in alum mixed with 200 ng/mouse pertussis toxin (Sigma-Aldrich, #70323-44-3). ELISA was performed to measure serum responses to antigen (see methods below) and the 3 best immunized animals were boosted with the same antigen mix. Four days later, splenocytes were fused with myeloma cells P3X63Ag8 (ATCC, #TIB-9) using Clon-aCell-HY Hybridoma Kit according to manufacturer's instructions (StemCell Technologies, #03800). Culture supernatants were screened using ELISA (see below) and antigen-reactive clones were expanded in RPMI-1640 complemented with 10% IgG-free Fetal Calf Serum (Sigma-Aldrich, #F1283) into roller bottles (Sigma-Aldrich, #CLS431344) at 37 °C. After 14 days, supernatants were harvested by centrifugation at 2500 rpm for 30 min and filtered (0.2 µm). Antibodies were purified by protein A affinity chromatography (AKTA pure) as described previously [33].

ELISA assays

Maxisorp microtiter plates (Dutscher, #055260) were coated with a total of 0.3 µg per well of LMW recombinant proteins in carbonate-bicarbonate buffer (pH 9.6) for 2 h at room temperature (RT). Free sites were blocked

by a 2-h incubation at RT with PBS 1% BSA. Plates were washed three times with PBS 0.05% Tween 20 (PBS-T) before being coincubated with serum, supernatants, or monoclonal antibodies at different concentrations (from 10^{-6} µg/mL to 10 µg/mL) for 1 h at RT. After five washes, goat anti-mouse IgG-Fc fragment HRP conjugated antibody (Bethyl, dilution 1:20,000, #A90-131P) was added for 1 h at RT followed by incubation with OPD (o-phenylenediamine dihydrochloride) revelation substrate for 10 min (Sigma-Aldrich, #P8287). Absorbances were analyzed at 492 vs 620 nm on an ELISA plate reader (Berthold).

Bio-layer interferometry

Biolayer interferometry assays were performed using Anti-Mouse Fc Capture biosensors on an Octet Red384 instrument (ForteBio, #18-5088). Monoclonal antibodies (10 µg/mL) were captured on the sensors at 25 °C for 1800 s. Biosensors were equilibrated for 10 min in PBS, 0.05% Tween 20, 0.1% BSA (PBS-BT) prior to measurement. Association was monitored for 1200 s in PBS-BT with LMW at a range of concentrations from 0.01 nM to 500 nM followed by dissociation for 1200 s in PBS-BT. Traces were reference sensor (sensors loaded with an unspecific mAb) subtracted and curve fitting was performed using a global 1:1 binding model in the HT Data analysis software 11.1 (ForteBio), allowing to determine K_D values.

Flow cytometry assays

mAb binding to whole bacteria was assessed by bacterial flow cytometry, as previously described [34]. Bacteria were fixed in 4% paraformaldehyde (PFA) for 30 min and resuspended in PBS and stained (10^6 bacteria/condition) using 5 µM Syto9 (Thermo Fisher Scientific, #S34854) in 0.9% NaCl for 30 min at RT. Bacteria were washed (10 min, 4000 g, 4 °C) and resuspended in PBS, 2% BSA and 0.02% Sodium Azide (PBA). Monoclonal antibodies were pre-diluted in PBA at 20 µg/mL and incubated with bacteria for 30 min at 4 °C. Bacteria were washed, and incubated with AF647 AffiniPure goat anti-mouse IgG (H + L) antibody or isotype control (Jackson ImmunoResearch, #115-605-003) for 30 min at 4 °C. After washing, bacteria were resuspended in sterile PBS. Flow cytometry acquisition was performed on a MacsQuant cytometer (Miltenyi) and analyzed on FlowJo software (BD Biosciences). Staining index was calculated by subtracting the Mean Fluorescence Intensity (MFI) of the isotype from the MFI of each condition with the anti-LMW mAbs, then divided by the MFI of the isotype.

Growth assays

Overnight *C. difficile* cultures were grown in TY broth and sub-cultured to an Optical Density at 600 nm (OD600nm) of 0.05 in 200 µL of BHISG in 96-well flat bottom plates (Merck, #Z707902) containing mAbs at 0.2 mg/mL. Bacterial growth was followed for 24 h or 18 h with OD600nm measurements every 30 min using GloMax Plate Reader (Promega). Anaerobiosis was maintained with a O₂ less sealing film (Sigma-Aldrich, #Z380059).

Sequence alignments

Sequence alignments of the LMW of five clinical ribotypes (LMW-RT001, LMW-RT002, LMW-RT014, LMW-RT027, LMW-RT078) have been performed using by ClustalOmega software. Fully conserved residues are indicated by (*), groups of strongly similar properties by (:) and groups of weakly similar properties by (.).

Statistical analysis

Growth and ELISA assays values were analyzed in Prism 8.0 (GraphPad, San Diego, CA). Statistical analysis was performed using two-way ANOVA test. A p value < 0.05 was considered significant.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13099-023-00592-7>.

Additional file 1: Figure S1. Comparison of two immunization protocols using recombinant LMWs. Mice were immunized following two different protocols termed “similarity” and “frequency”. **a** In the “Frequency” protocol, mice are immunized with LMWs in the order of their frequency in current CDI, and boosted with a mix of all five LMWs. In the “Similarity” protocol, mice are immunized with two highly similar LMW the same day, and boosted with a mix of all five LMWs. **b** Dose response of sera titers of immunized mice from the protocols depicted in (a) are measured by ELISA against the indicated LMW ribotype. Data are presented as mean values (±SD) for each group of mice (n = 5). ns: non-significant; *: p < 0.05. Black curves represent sera from naive mice prior immunization.

Acknowledgements

We thank Stéphane Petres (Production and Purification of Recombinant Proteins Facility, Institut Pasteur, 75015 Paris, France) for his advice on protein purifications.

Author contributions

Experimental design, LH, DS and PB; Conducting experiments, LH, BI, OG; Data analyses and discussions: LH, PE, FB, BD, LM, GG, DS and PB. Writing (original draft), LH, DS and PB; Writing (review and editing), all authors.

Funding

Work in the Bruhns’lab was supported by the Fondation Janssen Horizon (grant #2018-102415235), Institut Pasteur, Institut National de la Santé et de la Recherche Médicale (Inserm), Fondation pour la Recherche Médicale, Paris, France (Programme Equipe FRM grant EQU202203014631), Agence Nationale de la Recherche (ANR grant ANR-21-CE15-0027). LH is a doctoral fellow of

Sorbonne Université. DS was a recipient of a poste d’accueil 2017 Institut Pasteur—Assistance Publique des Hôpitaux de Paris (APHP). Work in the G. Gorochov’s team is supported by Institut National de la Santé et de la Recherche Médicale (INSERM), Sorbonne Université, Fondation pour la Recherche Médicale (FRM), Paris, France, program “Investissement d’Avenir” launched by the French Government and implemented by the Agence Nationale de la Recherche (ANR) with the reference COFIFERON ANR- 21-RHUS-08, by EU Horizon HLTH-2021-DISEASE-04 UNDINE project, by programme DIM Ile de France thérapie cellulaire et génique, by Fondation pour la Recherche Médicale, Paris, France (Programme Equipe FRM 2022) and by the Département Médico-Universitaire de Biologie et Génomique Médicales (DMU BioGen), APHP, Paris, France.

Declarations

Competing interests

Unrelated to the submitted work, P.B. received consulting fees from Regeneron Pharmaceuticals. The other authors declare no competing interests.

Received: 19 September 2023 Accepted: 20 December 2023

Published online: 19 January 2024

References

- Giles J, Roberts A. Clostridioides difficile: current overview and future perspectives. Adv Protein Chem Struct Biol. 2020. <https://doi.org/10.1016/bs.apcsb.2021.11.003>.
- Poxton IR, McCoubrey J, Blair G. The pathogenicity of Clostridium difficile. Clin Microbiol Infect. 2001;7:421–7.
- Herbert R, et al. Two-year analysis of Clostridium difficile ribotypes associated with increased severity. J Hosp Infect. 2019;103:388–94.
- Furuya-Kanamori L, et al. Comorbidities, exposure to medications, and the risk of community-acquired clostridium difficile infection: a systematic review and meta-analysis. Infect Control Hosp Epidemiol. 2015;36:132–41.
- Deshpande A, et al. Risk factors for recurrent Clostridium difficile infection: a systematic review and meta-analysis. Infect Control Hosp Epidemiol. 2015;36:452–60.
- Miller M, et al. Health care-associated clostridium difficile infection in Canada: patient age and infecting strain type are highly predictive of severe outcome and mortality. Clin Infect Dis. 2010;50:194–201.
- Couturier J, Davies K, Gateau C, Barbut F. Ribotypes and new virulent strains across Europe. In: Mastrantonio P, Rupnik M, editors. Updates on clostridium difficile in Europe. Cham: Springer International Publishing; 2018. p. 45–58.
- Oliveira Paiva AM, Friggen AH, Douwes R, Wittekoek B, Smits WK. Practical observations on the use of fluorescent reporter systems in Clostridioides difficile. Antonie Van Leeuwenhoek. 2022;115:297–323.
- Donnelly ML, et al. Development of a dual-fluorescent-reporter system in clostridioides difficile reveals a division of labor between virulence and transmission gene expression. mSphere. 2022;7:e0013222.
- Buckley AM, et al. Lighting up clostridium difficile: reporting gene expression using fluorescent lov domains. Sci Rep. 2016;6:23463.
- Freeman J, et al. The changing epidemiology of Clostridium difficile infections. Clin Microbiol Rev. 2010. <https://doi.org/10.1128/CMR.00082-09>.
- Kuehne SA, et al. Importance of Toxin A, Toxin B, and CDT in virulence of an epidemic clostridium difficile strain. J Infect Dis. 2014;209:83–6.
- Péchiné S, Denève-Larrazet C, Collignon A. Clostridium difficile Adhesins. In: Roberts PM, Adam P, editors. Clostridium difficile. New York: Springer New York; 2016. p. 91–101. https://doi.org/10.1007/978-1-4939-6361-4_7.
- Kirk JA, et al. New class of precision antimicrobials redefines role of Clostridium difficile S-layer in virulence and viability. Sci Transl Med. 2017. <https://doi.org/10.1126/scitranslmed.aah6813>.
- Pantaléon V, et al. The clostridium difficile protease Cwp84 modulates both biofilm formation and cell-surface properties. PLoS ONE. 2015;10:e0124971.

16. Ní Eidhin DB, O'Brien JB, McCabe MS, Athié-Morales V, Kelleher DP. Active immunization of hamsters against *Clostridium difficile* infection using surface-layer protein. *FEMS Immunol Med Microbiol*. 2008;52:207–18.
17. Calabi E, Calabi F, Phillips AD, Fairweather NF. Binding of *Clostridium difficile* surface layer proteins to gastrointestinal tissues. *Infect Immun*. 2002;70:5770–8.
18. Fagan RP, et al. Structural insights into the molecular organization of the S-layer from *Clostridium difficile*. *Mol Microbiol*. 2009;71:1308–22.
19. Merrigan MM, et al. Surface-Layer Protein A (SlpA) is a major contributor to host-cell adherence of *Clostridium difficile*. *PLoS One*. 2013. <https://doi.org/10.1371/journal.pone.0078404>.
20. Eidhin DN, Ryan AW, Doyle RM, Walsh JB, Kelleher D. Sequence and phylogenetic analysis of the gene for surface layer protein, slpA, from 14 PCR ribotypes of *Clostridium difficile*. *J Med Microbiol*. 2006;55:69–83.
21. Bruxelle JF, et al. Immunogenic properties of the surface layer precursor of *Clostridium difficile* and vaccination assays in animal models. *Anaerobe*. 2016;37:78–84.
22. Mizrahi A, Bruxelle JF, Péchiné S, Le Monnier A. Prospective evaluation of the adaptive immune response to SlpA in *Clostridium difficile* infection. *Anaerobe*. 2018;54:164–8.
23. Macdonald LE, et al. Precise and in situ genetic humanization of 6 Mb of mouse immunoglobulin genes. *Proc Natl Acad Sci*. 2014;111:5147–52.
24. Murphy AJ, et al. Mice with megabase humanization of their immunoglobulin genes generate antibodies as efficiently as normal mice. *Proc Natl Acad Sci*. 2014;111:5153–8.
25. Calabi E, et al. Molecular characterization of the surface layer proteins from *Clostridium difficile*. *Mol Microbiol*. 2001. <https://doi.org/10.1046/j.1365-2958.2001.02461.x>.
26. Chen J, et al. B cell development in mice that lack one or both immunoglobulin kappa light chain genes. *EMBO J*. 1993;12:821–30.
27. Péchiné S, et al. Immunological properties of surface proteins of *Clostridium difficile*. *J Med Microbiol*. 2005;54:193–6.
28. Lanzoni-Mangutchi P, et al. Structure and assembly of the S-layer in *C. difficile*. *Nat Commun*. 2022. <https://doi.org/10.1038/s41467-022-28196-w>.
29. Knight DR, et al. Genomic epidemiology and transmission dynamics of recurrent *Clostridioides difficile* infection in Western Australia. *Eur J Clin Microbiol Infect Dis*. 2023;42:607–19.
30. Clifford McDonald L, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. *N Engl J Med*. 2015. <https://doi.org/10.1056/NEJMoA051590>.
31. Loo VG. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N Engl J Med*. 2006;354(20):2200.
32. Knight DR, et al. Genomic epidemiology and transmission dynamics of recurrent *Clostridioides difficile* infection in Western Australia. *Eur J Clin Microbiol Infect Dis*. 2023. <https://doi.org/10.1007/s10096-023-04569-x>.
33. Balbino B, et al. The anti-IgE mAb omalizumab induces adverse reactions by engaging Fcγ receptors. *J Clin Invest*. 2020;130:1330–5.
34. Fadlallah J, et al. Synergistic convergence of microbiota-specific systemic IgG and secretory IgA. *J Allerg Clin Immunol*. 2019;143:1575–1585.e4.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.