Comparison of *Clostridium sordellii* toxins HT and LT with toxins A and B of *C. difficile*

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Summary. *Clostridium sordellii* produces two toxins, designated HT (haemorrhagic toxin) and LT (lethal toxin), that are similar to toxins A and B of *C. difficile*. The physicochemical properties of toxins HT and A were remarkably similar. The specific biological activities of toxin HT were almost the same as those of toxin A, and their NH₂-terminal sequences shared close homology. The properties of toxins LT and B were similar, as were their NH₂-terminal sequences, but toxin B was much more cytotoxic than toxin LT. Immunodiffusion analysis with specific antibodies showed that although toxins B and LT shared major antigenic determinants, each had unique epitopes. The results suggest that toxins B and LT have diverged more than toxins A and HT. Immunoblotting with antibodies to the toxins of *C. difficile* showed that toxins HT and LT had common antigenic determinants.

Introduction

Several studies have implicated *Clostridium sordellii* as a cause of diarrhoea and enterotoxaemia in domestic animals,^{1–3} and, more recently, as an agent of toxic shock-like syndrome in man.^{4,5} This species was once suspected to be the cause of pseudomembranous colitis (PMC) in man because the cytotoxicity of faecal filtrates from PMC patients was neutralised by *C. sordellii* antitoxin.^{6–8} However, *C. sordellii* could not be isolated from the faeces of patients with PMC. This discrepancy was clarified when *C. difficile* was isolated from faecal samples of patients with PMC,^{9–11} and it was shown that the toxins produced by this organism are neutralised by *C. sordellii* antitoxin.^{6,8,12,13} Two toxins, A and B, have been purified from culture supernate of toxigenic strains of *C. difficile*.^{14–16} Both are large proteins that are lethal to animals and cytotoxic. Toxin A is a potent enterotoxin that produces a haemorrhagic fluid response in the rabbit ileal loop assay.^{17–19}

C. sordellii produces two toxins that are similar to toxins A and B, which explains why *C. sordellii* antitoxin neutralises the toxins of *C. difficile*. The production of two distinct toxins by *C. sordellii* was first described by Arseculeratne *et al.*,²⁰ who extracted a haemorrhagic toxin from sporulating cells and an oedema-producing toxin from vegetative cells. The oedema-producing toxin was more lethal than the

haemorrhagic toxin and the toxins are now referred to as LT (lethal toxin) and HT (haemorrhagic toxin) respectively. We have already described the purification of toxin HT by ultrafiltration and immuno-affinity chromatography with a monoclonal antibody (MAb) to toxin A, and have shown that toxin HT has biological activities and immunological properties similar to those of toxin A.²¹ Popoff has purified toxin LT and shown that it is immunologically related to toxin B.²² The toxins produced by *C. difficile* and by *C. sordellii* have similar physicochemical as well as immunological and biological properties but they are not identical. Therefore, it was of interest to compare the properties of these toxins in more detail.

Materials and methods

Protein determination

Protein concentration was estimated by the method of Bradford²³ with the BioRad Protein Assay Kit (BioRad Laboratories, Richmond, CA, USA). Bovine γ -globulin (BioRad) was the standard.

Bacterial strains and medium

C. difficile VPI 10463 (Tox⁺), *C. sordellii* VPI 9048 (Tox⁺), VPI 2013 (Tox⁻) and VPI 7319 (Tox⁻) were obtained from the culture collection of the Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University (Blacksburg) and were identified by L. V. Holdeman, E. P. Cato and W. E. C. Moore by methods described in the Virginia Polytechnic Institute Anaerobe Laboratory Manual.²⁴

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Cells were grown in brain heart infusion broth in 2-L dialysis flasks for 72 h at 37°C as described for the production of *C. botulinum* toxin.²⁵

Antisera

Antisera against crude *C. sordellii* VPI 9048 culture filtrate was raised in rabbits as described before.²¹ Briefly, toxoid was prepared by incubating crude culture filtrate in formaldehyde (0.1% v/v final concentration) for 3 h at 37°C. Female New Zealand White rabbits (Hazleton Research Animals, Denver, PA, USA) were given 2.5 ml of formalinised culture filtrate mixed with an equal volume of Freund's incomplete adjuvant (Sigma) by subcutaneous injection each week. Antitoxin reached maximum titres 24 weeks after the first injections. At that time the rabbits were exsanguinated by cardiac puncture. To obtain affinity-purified antibodies against toxin HT, a preparation of toxin HT purified to homogeneity (as described below) was coupled to Affi-Gel 10 (BioRad) as recommended by the manufacturer; 0.5 mg of toxin was bound per ml of gel. *C. sordellii* rabbit antiserum (1 ml) was dialysed against 0.05 M Tris hydrochloride buffer, pH 7.5, containing 0.15 M NaCl (TBS). The dialysed material was applied to the column (0.5 × 10 cm) of toxin HT-Affi-Gel 10, and the column was washed with TBS to remove unbound material. Bound antibodies were eluted by adding 2 ml of 0.1 M glycine-0.15 M NaCl (pH 3.0) to the column and washing with TBS. The eluate, designated affinity-purified toxin HT antibody preparation, was washed twice with TBS (4 ml per wash) on a Minicon-B15 concentrator (Amicon Corp., Lexington, MA, USA) and concentrated to 0.5 mg/ml.

Rabbit and goat antisera against crude *C. difficile* VPI 10463 culture filtrate were produced as described previously.²⁶ Affinity-purified goat antibodies and MAb were also prepared as previously described.²⁷

Purification of toxin HT

Toxin HT was purified from the culture filtrate of *C. sordellii* VPI 9048 by ultrafiltration on an XM-300 membrane (Amicon) and by immuno-affinity chromatography with MAb PCG-4, a MAb to toxin A, coupled to Affi-Gel 10 (BioRad) as described previously.²¹ A partially purified preparation of toxin HT was obtained as described by Yamakawa *et al.*²⁸

Partial purification of toxin LT

Partially purified toxin LT was obtained by a procedure similar to that described by Yamakawa *et al.*²⁸ *C. sordellii* VPI 9048 culture filtrate (100 ml) was concentrated to 10 ml by ultrafiltration on an XM-100 membrane (Amicon). The concentrated culture filtrate was equilibrated with 50 mM Tris-HCl buffer, pH 7.5, containing 22 mM NaCl, and applied to a column (0.5 × 10 cm) containing 1 ml of DEAE-Sepharose

CL-6B (Sigma). The gel was washed with 25 bed volumes of 50 mM Tris-HCl, pH 7.5, containing 22 mM NaCl. Toxin LT was eluted with three bed volumes of 50 mM Tris-HCl, pH 7.5, containing 25 mM NaCl. The sample was passed through a column containing PCG-4 MAb fixed to Affi-Gel 10 to remove any contaminating HT. The toxin LT preparation was dialysed against TBS, concentrated to 0.2 ml with a Minicon-B15 concentrator and stored at 4°C.

Polyacrylamide gel electrophoresis (PAGE)

Non-denaturing PAGE was done in a discontinuous system as described before.²⁹ Toxin samples were mixed with bromophenol blue-glycerol and loaded on to 4% stacking-7.5% running gels. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was done according to the general procedures of Laemmli.³⁰ Toxin samples containing SDS 2.5% and 2-mercaptoethanol 5% were heated at 100°C for 2 min, mixed with bromophenol blue-glycerol, and applied to 4% stacking-7.5% running gels containing SDS 0.1%. Electrophoresis was done at 60 V at room temperature in 0.025 M Tris-0.192 M glycine buffer, pH 8.3, for non-denaturing PAGE and the same buffer containing SDS 1% for denaturing PAGE. LT preparation gels, stained with Coomassie Brilliant Blue R-250, were scanned with a densitometer (model 620 video densitometer; BioRad) in the reflectance mode to determine the proportion of each protein band in the partially purified toxin.

Immunoblotting

After electrophoresis, the samples were blotted on to nitrocellulose membranes at 230 mA for 16 h in 0.025 M Tris-0.192 M glycine buffer, pH 8.3, containing methanol 20%.²⁹ The nitrocellulose membranes were rinsed with TBS and blocked for 1 h at room temperature with TBS containing sodium caseinate 0.5%. The membranes were incubated with the specific antibody preparation (diluted 1 in 100 in TBS) for 4 h at room temperature. After incubation, the membranes were rinsed three times (20 min/rinse) and incubated overnight at room temperature in rabbit anti-goat or goat anti-rabbit IgG-horseradish peroxidase conjugate (Sigma) diluted 10³ in TBS. The membranes were rinsed three times in TBS, and blotted proteins were detected with the 4-chloro-1-naphthol reagent (Sigma).

Enzyme-linked immunosorbent assay (ELISA)

An indirect ELISA for the detection of toxin HT was done as described previously for the detection of toxin A of *C. difficile*.³¹ MAb PCG-4 was used as the detecting antibody. The amount of toxin HT in the partially purified HT preparation was calculated from the linear portion of the ELISA standard curve obtained with known concentrations of toxin HT.

Crossed immuno-electrophoresis and immunodiffusion analysis

Crossed immuno-electrophoresis¹⁵ and Ouchterlony double-immunodiffusion analysis²¹ were done as described previously.

Amino-acid analysis

Amino-acid composition of purified toxin HT was determined in triplicate on 24-, 48-, and 72-h hydrolysed samples by the method of Spackman *et al.*³² as described before.²⁹ The tryptophan content was determined spectrophotometrically by the Edelhoch technique.³³

Amino-acid sequence

The amino-terminal sequence of toxins HT and LT were determined by the method of Matsudaira,³⁴ at the Protein Sequencing Center of the University of Virginia, Charlottesville, VA. Toxin preparations consisted of affinity-purified HT (30 µg) and partially purified LT (50 µg).

Biological assays

Cytotoxicity, lethality, rabbit intestinal loop and neutralisation assays were performed by published methods.^{15, 18, 35, 36}

Haemagglutination assay

Haemagglutinating activity of toxin HT was determined as described previously.³⁷

Results

Toxin purification and characterisation

Toxin HT was removed from the concentrated culture filtrate by the MAb to toxin A of *C. difficile* coupled to Affi-Gel 10. The toxin HT that eluted from the PCG-4 MAb column appeared to be homogeneous by crossed immuno-electrophoresis (fig. 1). The preparation of toxin HT gave one major band and a minor band on non-denaturing PAGE (fig. 2) as reported previously.²¹ Toxin HT migrated on SDS-PAGE as one major band with an estimated mol. wt of 300 000 and several faster migrating bands (fig. 3). The NH₂-terminal sequence of toxin HT was determined and compared with the deduced NH₂-terminal sequence of toxin A.³⁸ The NH₂-terminal sequence of toxins A and HT are almost identical (table I).

Toxins HT and LT in *C. sordellii* culture filtrate were separated on DEAE-Sepharose CL-6B. The DEAE-Sepharose CL-6B fraction containing toxin LT was passed through a MAb PCG-4 Affi-Gel 10 column to remove any contaminating toxin HT. Several immunoprecipitin arcs were observed by

Fig. 1. Analysis of toxin preparations by crossed immuno-electrophoresis: (a) *C. sordellii* VPI 9048 culture filtrate (75 µg); (b) toxin HT purified by immuno-affinity chromatography on MAb PCG-4-AffiGel 10 (2.5 µg); (c) partially purified toxin LT (15 µg). The upper portion of each plate contained 250 µl of rabbit antiserum raised against a culture filtrate from *C. sordellii* VPI 9048.

crossed-IEP (fig. 1). Analysis by PAGE revealed at least four protein bands in the partially purified toxin LT preparation (fig. 2). A major band with an estimated mol. wt of 260 000 and several faster migrating bands were observed after SDS-PAGE of the LT preparation (fig. 3). This major band appeared as a doublet and represented 80% of the protein in the partially purified toxin LT preparation, as determined by densitometric analysis. The NH₂-terminal sequence of this 260 000-mol. wt protein band was determined and revealed homology with the sequence recently published for toxin B^{39, 40} (table I).

Immunodiffusion analysis with affinity-purified toxin A antibody revealed a reaction of partial identity between toxins A and HT (fig. 4). Similarly, affinity-

Fig. 2. (A) PAGE and immunoblot (IB) analysis with affinity-purified antibody to toxin A: (1) *C. sordellii* VPI 9048 culture filtrate (200 µg); (2) purified HT (10 µg); (3) partially purified LT (12 µg). (B) PAGE and IB analysis of *C. sordellii* culture filtrate (cf. 200 µg) with the affinity-purified antibody to toxin B.

Table I. Amino-terminal sequence of toxins A, B, HT and LT

Toxin	Amino-terminal sequence															
	1				5				10				15			
A*	Ser	Leu	Ile	Ser	Lys	Glu	Glu	Leu	Ile	Lys	Leu	Ala	Tyr	Ser	Ile	Arg
HT	Ser	Leu	Ile	Ser	Lys	Ser	Glu	Leu	Ile	Lys	Leu	Ala	Tyr	Ser	Ile	Pro
B†	Ser	Leu	Val	Asn	Arg	Lys	Gln	Leu	Glu	Lys	Met	Ala	Asn	Val	Arg	Phe
LT	Arg	Leu	Val	Asn	Lys	Arg	Gln	Leu	Gln	Lys	Met	Ala	Tyr	Val	Lys	Asp

* Deduced from the nucleotide sequence.³⁸† Deduced from the nucleotide sequence.⁴⁰ The amino-terminal Ser was reported as a Trp by Meador and Tweten.³⁹ **Fig. 3.** SDS-PAGE and immunoblot (IB) analysis with affinity-purified toxin A antibody: (1) purified HT (10 µg); (2) partially purified LT (12 µg). **Fig. 4.** Ouchterlony immunodiffusion analysis: (1) toxins A (T_A , 20 µg) and HT (20 µg) vs affinity-purified antibody to toxin A (T_A Ab); (2) toxins B (T_B , 30 µg) and LT (30 µg) vs affinity-purified antibody to toxin B (T_B Ab).

purified antibody to toxin B reacted with toxin LT and formed a precipitin line that showed partial identity with toxin B (fig. 4). The toxins were not immunoprecipitated by the heterologous antisera.

Immunoblot analysis

Under non-denaturing conditions, affinity-purified antibody to toxin A reacted with the purified toxin

HT, with HT in the culture filtrate (fig. 2), and with the major and minor bands of HT observed by SDS-PAGE (fig. 3). Toxin A antibody also reacted with toxin LT under both non-denaturing (fig. 2) and denaturing conditions (fig. 3), but was less reactive with LT than with HT. Similar results were obtained when toxins A and B reacted with affinity-purified antibodies to toxin HT. Affinity-purified antibody to toxin B reacted with the bands corresponding to toxins HT and LT in the culture filtrate, and the reaction was stronger with LT (fig. 2). Under denaturing conditions, both toxins and their minor bands were recognised by toxin B antibodies, and the reaction patterns were very similar to those shown with antibody to toxin A. The antibodies to toxins A and B did not react with any antigen in the culture filtrates from the non-toxicogenic strains (VPI 2013 and 7319) of *C. sordellii*.

Amino-acid analysis

The amino-acid comparison of HT is shown in table II, and compared with those previously published for toxins A, B and LT.^{22, 29, 41}

Biological activity

Immuno-affinity purification of toxin HT resulted in partial inactivation. However, with an HT-ELISA we were able to quantify HT in the partially purified preparation, and thus determine the specific activities of the native toxin HT. Toxins HT and LT had specific activities in the tissue-culture assay with Chinese Hamster Ovary-K1 (CHO-K1) cells of 6.6×10^4 CU (cytotoxic units)/mg and 6.2×10^5 CU/mg, respectively. The specific activities of the toxins in the mouse lethality assay were 1.3×10^4 LD 100/mg for HT and 2.0×10^5 LD 100/mg for LT. A comparison of the biological activities of the *C. difficile* and *C. sordellii* toxins is presented in table III. We also determined the cytotoxic activity of toxins HT and LT on the mouse teratocarcinoma cell lines F9 and P19. Toxin HT was approximately 100-fold more active on these cell lines than on the CHO-K1 cells. No increase in the activity of toxin LT was observed.

Affinity-purified antibody to toxin A neutralised the cytotoxicity, mouse lethality and enterotoxicity of

Table II. Amino-acid composition of toxins A, B, HT and LT

Amino acids	Content (mol.%)			
	T _A *	HT*	T _B †	LT‡
Asp/Asn	15.0	7.8	14.7	13.4
Thr	5.1	7.6	5.1	5.9
Ser	7.6	8.7	7.5	6.1
Glu/Gln	8.9	6.6	11.5	10.3
Pro	2.1	3.7	2.6	2.9
Gly	7.6	7.3	10.7	8.3
Ala	5.9	5.2	4.5	7.3
Val	5.4	4.5	6.0	6.1
Met	1.0	1.8	0.3	2.5
Ile	8.4	9.8	8.6	7.9
Leu	8.7	10.7	8.0	6.7
Tyr	4.4	5.8	2.8	4.5
Phe	5.4	6.8	5.0	4.9
His	1.8	1.9	1.4	1.8
Lys	8.7	9.0	6.4	6.9
Arg	3.1	2.7	2.5	2.7
Trp	1.2	1.5	1.9	ND
Cys	0.8	ND	0.5	1.3

ND, not determined.

* Toxins A and HT were purified by immuno-affinity chromatography on PCG-4 antibody Affi-Gel 10.^{21,29}† Reported by Lyerly *et al.*⁴¹‡ Reported by Popoff.²²

toxin HT, but not the biological activities of the partially purified toxin LT. Affinity-purified antibody to toxin B neutralised the cytotoxicity and lethal activities of toxin LT, but did not neutralise the biological activities of HT. None of the individual highly purified antibodies to either toxin A or B totally neutralised the cytotoxic or lethal activities of the *C. sordellii* culture filtrate that contained both toxins. However, a combination of toxin A and toxin B antibodies did neutralise the cytotoxicity and lethality of the culture filtrate, indicating that the toxicity of the culture filtrate of *C. sordellii* is due to the toxins HT and LT. Antibodies to toxin A (MAb PCG-4 or the affinity-purified antibody) neutralised all of the enterotoxigenic activity of *C. sordellii* culture filtrate.

Haemagglutination

Toxin HT agglutinated rabbit erythrocytes, although the titres were approximately 100-fold lower than those reported for toxin A.³⁷

Discussion

The approach we used to obtain a partially purified preparation of toxin LT was similar to that used for the purification of *C. difficile* toxin B.¹⁵ The anion exchange step enabled separation of toxin HT from LT and adsorption of HT with the MAb gel achieved complete removal of HT from the LT preparation. The cytotoxic and lethal activities of this preparation were completely neutralised by antibody to toxin B; this would not have been true if HT had been present.

Table III. Biological activities of toxins A, B, HT and LT

Toxin	Tissue culture dose	Lethal dose in mice	Enterotoxigenic dose in rabbit ileal loop
A*	10 ng	50–90 ng	1 µg
HT†	15 ng	75 ng	2 µg
B*	0.0002–0.001 ng	50 ng	Negative
LT‡	1.6 ng	5 ng	Negative

* Lyerly *et al.*^{18,41} and Sullivan *et al.*¹⁵† Doses are based on the amount of toxin HT in the partially purified preparation as determined by ELISA.^{21,31}‡ Popoff reported values of 16 and 2.5 ng for TCD and LD, respectively. A low, non-haemorrhagic enterotoxigenic response in guinea-pig intestinal loops was also reported.²²

Purified toxin HT had a major and a minor band when analysed by PAGE under non-denaturing conditions. The minor band reacted with antibodies to toxin A and was detected in the culture filtrate;²¹ therefore, it was not a contaminant and did not result from the purification procedure. Toxins HT and A have mol. wts in excess of 300 000, as determined by SDS-PAGE.^{21,29} The gene for toxin A has been sequenced, and encodes for a polypeptide with a calculated mol. wt of 308 103,³⁸ which supports the observations from SDS-PAGE. Toxins LT and B are also extremely large proteins with mol. wts of about 250 000.^{22,29,39} These observations are supported by the finding that the gene for toxin B is 7.1 kb long and codes for a polypeptide with deduced mol. wt of 269 696.⁴² Faster-migrating bands were observed when the toxins HT and LT were denatured and analysed by SDS-PAGE. Immunoblot analyses revealed that these minor bands reacted with antibodies specific to the toxins of *C. difficile*. The minor bands generated from toxin HT have also been shown to react with MAb PCG-4.²¹ The appearance of similar minor bands is characteristic of toxins A and B of *C. difficile* and may result from proteolysis.²⁹

As a difference has been demonstrated between the iso-electric points of toxins A and HT,²¹ we compared the amino-acid composition of the two toxins. This revealed a two-fold difference in the amounts of aspartic acid/asparagine, with only minor differences in the amounts of other amino-acid residues, which may explain the difference in pI. However, the two toxins had similar specific activities (table III), indicating that, despite the compositional differences, the biological activities were not significantly changed. Like toxins A and HT, toxins B and LT have relatively high amounts of hydrophobic amino acids, which may explain the formation of aggregates in the culture filtrate as shown by immunoblotting (fig. 2).

Toxin LT, analysed by SDS-PAGE, appeared as a doublet band, which is analogous to that seen with toxin B.⁴¹ However, the amino-terminal sequence of this band was unambiguous, which indicates that it is a single protein. Furthermore, the amino-terminal sequence revealed homology with the sequence pub-

lished for toxin B,^{39,40} which clearly supports its identity. The fact that most of the amino-acid changes observed would involve a single nucleotide change further supports the fact that these toxins are closely related. High levels of homology were also observed between the amino-terminal sequences of toxins A and HT.

Affinity-purified toxin HT retained its biological activities, which were similar to those observed with toxin A and included cytotoxicity (cell rounding), lethality in mice, and enterotoxicity. Elution of HT from the antibody column with $MgCl_2$ resulted in partial inactivation of the toxin, but we were able to quantitate HT in a partially purified preparation by an HT-ELISA, and thus estimate its specific activities in its native state. With this approach, we found that the specific activities of toxin HT in the biological assays are very similar to those of toxin A (table III).

As with toxin B, toxin LT was shown to be cytotoxic and lethal but not enterotoxic. Toxin B is at least 1000-fold more cytotoxic than LT, whereas LT is 10-fold more active in the mouse lethality assay. These differences in biological activity may result from variations in the structure of the toxins as noted in this paper (e.g., incomplete immunological cross-reactivity and only 59% homology in their amino-terminal sequences).

Antibodies to either toxin A or toxin B alone did not neutralise all of the cytotoxic or lethal activities of the *C. sordellii* culture filtrate. However, antibodies to both toxins together completely neutralised these activities. This indicates that toxins HT and LT are responsible for all the cytotoxic and lethal activities of the *C. sordellii* culture filtrate. Affinity-purified antibody to toxin A, as well as the MAb PCG-4, completely neutralised the enterotoxicity of the *C. sordellii* culture filtrate, indicating that this activity is due solely to toxin HT just as the enterotoxicity of the *C. difficile* culture filtrate is due solely to toxin A.¹⁹

In the past we have attempted to purify toxin HT by the receptor-affinity column procedure that has been described for the purification of toxin A.^{21,43} In this procedure, toxin A binds to the trisaccharide receptor Gal α 1-3 Gal β 1-4 GlcNAc on bovine thyroglobulin at 4°C, and is then eluted by increasing the temperature to 37°C.⁴³ Although this approach resulted in only partial purification of toxin HT, the results did show that toxin HT binds to this trisaccharide.²¹ Further evidence indicating that toxins A and HT share similar receptor-binding sites came from neutralisation experiments with the MAb PCG-4. This MAb neutralises the enterotoxic activities of toxin HT and toxin A,^{21,29} and has been shown to react with a recombinant peptide representing the region of toxin A that binds to the trisaccharide Gal α 1-3 Gal β 1-4 GlcNAc.⁴⁴ Moreover, DNA from the toxigenic *C. sordellii* strain (VPI 9048) hybridised with a 2.1-kb probe encoding the fragment of the toxin A gene that is responsible for the binding of toxin A to the trisaccharide sequence.⁴⁴ Cytotoxicity studies with

F9 and P19 cell lines provided additional evidence indicating that the receptors for toxins A and HT are very similar. These cells are more sensitive to toxin A, and the increase in sensitivity is due to the expression on the cell surface of the trisaccharide to which toxin A binds.³⁶ As with toxin A, toxin HT has increased cytotoxic activity in these cell lines.

No precipitation of either HT or LT was observed in the Ouchterlony double immunodiffusion test with the heterologous antibody preparation. Cross-neutralisation experiments showed that the antigenic determinants involved in neutralisation were not shared by these toxins. Nevertheless, the more sensitive immunoblot assay did show some cross-reactivity. Affinity-purified antibody to toxin A reacted strongly with HT and also showed some reactivity with LT. Similarly, affinity-purified antibody to toxin B showed a strong reaction with toxin LT but also reacted weakly with HT. The data support the presence of shared antigenic determinants between toxins HT and LT, but indicate a lower binding affinity of the heterologous antibodies to the cross-reactive epitopes. We reported previously that the MAb PCG-4, which appears to attach to the receptor binding region of toxin A, reacts with toxin HT, but does not react with toxin B or LT.^{21,29}

Immunological cross-reactivity between toxins produced by other organisms has been documented previously. The enterotoxins produced by *Staphylococcus aureus* comprise a group of proteins that once were regarded as antigenically distinct. However, varying degrees of immunological relatedness have been shown between these toxins.⁴⁵ A similar phenomenon is found with the shiga-like toxins produced by some strains of *Escherichia coli*. These toxins have been designated shiga-like toxin SLT-I and SLT-II and they have similar biological activities and physicochemical properties.⁴⁶ In addition, SLT-I and SLT-II share short regions of high amino-acid sequence homology (70–100%) with an overall homology of 56%; however, the toxins are not cross-neutralised by heterologous antiserum.^{46,47}

As with the SLTs, *C. difficile* toxins A and B are not cross-neutralised by heterologous antiserum,³⁵ however, comparison of the deduced amino-acid sequences of toxins A and B revealed regions of homology between these toxins⁴⁰ (J. L. Johnson, personal communication) which further supports our observations with the toxins of *C. sordellii*.

In conclusion, the findings presented here support previous observations of the relationship between the toxins of *C. difficile* and *C. sordellii*, and suggest greater divergence between toxins B and LT than between toxins A and HT. Further study of these antigenic and functional differences may provide a better understanding of these toxins.

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