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Lectin Array Analysis of Purified Lipooligosaccharide: A Method for the Determination of Molecular Mimicry

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Abstract

Surface glycosylation of bacteria is involved in many critical host-microbe interactions. Lectin arrays consisting of diverse carbohydrate binding proteins have proven to be an important tool for evaluating a wide variety of glycosylation, including that present on whole bacteria. However, assessing glycosylation on whole bacteria using lectin arrays may not reflect bacterial glycosylation, but interactions between bacteria and the glycosylation present on lectins. The lipooligosaccharide of *Campylobacter jejuni* NCTC 11168 and 81-176 are known to mimic the human monosialylated gangliosides. This molecular mimicry by *C. jejuni* can result in the post infection sequelae Guillain–Barré syndrome. Using *C. jejuni* as a model system and a discreet lectin and antibody array, a method, applicable to many organisms has been developed and validated by to screening of the purified lipooligosaccharide of *C. jejuni* for molecular mimicry to monosialylated gangliodises. In case of *C. jejuni,* knowing whether clinically important bacterial strains are capable of inducing severe autoimmune responses may aid in prevention and/or early diagnosis of debilitating post infection conditions.

Keywords: Lipooligosaccharide; Glycosylation; Lectin arrays

Introduction

Bacterial surface glycosylation is involved in many critical and diverse host-microbe interactions including adherence and immune modulation [1]. The analysis of bacterial surface glycosylation traditionally has been performed using nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS) and blotting based techniques [2-7]. Both NMR spectroscopy and MS are powerful techniques, but require relatively large quantities of highly purified glycan for structural analysis. Blotting methodologies, such as lectin blotting [7], have the advantage of only requiring small amounts of partially purified glycosylated protein or lipid for analysis, however only a single lectin interaction can be assessed per blot and the results can be ambiguous due to the difficulties in ensuring equimolar loading of test compounds into individual wells on the gel. In all cases these methodologies only provide low-throughput capabilities.

Lectin arrays consisting of diverse carbohydrate binding proteins covalently immobilised on glass microarray slides, have proven to be an important tool for evaluating cell surface glycosylation on whole bacteria and eukaryotic cells [1,8-10], eliminating the need to purify surface glycoproteins and lipids prior to analysis. However, assessing glycosylation on whole bacteria using lectin arrays may not be an accurate reflection of the bacterial glycosylation. Lectins are glycoproteins [11,12], therefore when immobilized on microarray slides they may themselves act as receptors for bacterial carbohydrate binding proteins. That is, bacterial glycan recognising adhesins [13-17] may interact with carbohydrate structures present on immobilised lectins rather than lectins recognising bacterial glycoconjugates. In order to overcome this potentially significant limitation, we report here the analysis of semi-purified bacterial glycan using a discrete lectin array that requires only a small quantity of product for accurate, quick, and reproducible glycan structure determination. As the model systems for lectin array-based glycan structure determination we assessed both purified and crudely isolated *C. jejuni* lipooligosaccharide (LOS).

Campylobacter jejuni is a Gram-negative spiral rod bacteria that synthesizes both *N*-linked and *O*-linked glycans [18]. The strains of *C. jejuni* with that produce sialylated LOS (biosynthesis cluster types A, B, C, M and R) are capable of causing an autoimmune response against human gangliosides. This autoimmune response occurs in approximately 1:1000 people infected with *C. jejuni* leading to the development of the debilitating and life-threatening diseases Guillain–Barré syndrome (GBS) or Miller Fisher Syndrome (MFS) [19-21]. Our novel approaches to glycan structure identification permitted the elucidation of a clinically important ganglioside structures involved in molecular mimicry, the basis for the development of GBS.

Materials and Methods

Bacterial strains and growth conditions

The original isolate of *C. jejuni* NCTC 11168 (11168-O) previously characterized [22] was kindly supplied by D.J. Newell (Veterinary Laboratories Agency, Weybridge, UK). The human isolates *C. jejuni* 81-176 was donated by James G. Fox (Massachusetts Institute of Technology, Cambridge, Massachusetts, USA) and *C. jejuni* 224 was obtained from the Royal Melbourne Institute of Technology (Melbourne, Vic., Australia). *C. jejuni* were grown on blood agar, composed of Columbia agar containing 5 % (v/v) defibrinated horse blood and Skirrow's antibiotic supplement (Oxoid), under microaerobic conditions (5 % O_2, 10 % CO_2 and 85 % N_2) at 37°C for 48 h.

Lipooligosaccharide preparations

Crude LOS preparation: Blood agar-grown bacteria were harvested in 1 mL of sterile water, washed once in 1 mL of sterile water, and lysed by heating. Prior to lysis, samples were adjusted for bacterial number by OD_{600} measurement of bacterial suspensions. Mini-preparations of LOS were prepared by treating the whole-cell extracts with proteinase

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K as described previously [23]. The LOS mini-preparations from single colonies were prepared by collecting and washing cells in 40 µL of sterile water followed by heat lysis. These preparations were diluted 10-fold prior to gel electrophoresis or lectin array analysis.

Purified LOS preparation: *C. jejuni* LOS was purified by subjecting the *C. jejuni* cell biomass to hot phenol-water treatment using 90 % (v/v) aqueous phenol at 65°C for 10 min [24], followed by enzymatic treatment as previously described [25]. The LOS preparations were adjusted to 15 µg/µL with distilled water prior to gel electrophoresis or lectin array analysis.

Electrophoretic analyses

Equal quantities of either LOS mini-preparations or purified LOS (~15 µg) were resolved on SDS-PAGE (5.5 % (w/v) and 10 % (v/v) stacking and separating acrylamide gels, respectively) containing 6 M urea and 0.3 mM tricine (Tricine-SDS-PAGE) as previously described [26]. Following electrophoresis at 20 V for 1 h to maximize stacking and then at 200 V for 30 min, gels were fixed and carbohydrate bands visualised by silver staining [27].

Lectin and western blotting

In addition to silver staining, fractionated *C. jejuni* LOS was electrotransferred from Tricine SDS-PAGE gels to Pall® PVDF membranes using a Trans-Blot SD Semi-Dry Transfer Cell (Bio-Rad) at 30 V for 60 min. Membranes were subsequently probed using either horseradish peroxidase (HRP)-conjugated CTB (Cholera toxin subunit B) (3 µg/mL), HRP-conjugated PNA (Peanut Agglutinin) (5 µg/mL), or HRP-conjugated anti-GM_1 ganglioside IgG (diluted 1:3000) in PBS. Membranes were developed SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) according to the manufacturer's instructions.

Primer Name	Primer Sequence	Tm
ORF6 F	GTAGTAGATGATTTGGGTAATGATAAA	47°C
ORF6 R	ATAGAATTGCTATTTACATGCTGG	
ORF8 F	CCTTTGATAATCCCTGAAATAGGT	50°C
ORF8 R	TCCTTTGCACTTATACCACCTT	
ORF5/10 F	GGTGTTATAGGATATAATGATTGTACTGATGG	49°C
ORF5/10 R	CCTCTGTTGTATCTATATCCAAACTAGC	
ORF12 F	GCCACAACTTTCTATCATAATCCCGC	50°C
ORF12 R	CGCCATAACTCAAACGCTCATCTATT	
ORF14 F	GCTAGAACACCCTAAAGTGACTAA	47°C
ORF14 R	TGGCACTAAATTGTAATAAATGGC	
ORF16 F	AGATCTGCAGCGTTTAGTGATTATTTTAG	47°C
ORF16 R	TACTCCAGATCCCCTATCGTCTC	

Table 1: Primers used in this study.

Lectin/ Antibody	Specificity	11168 [P]	11168 [C]	81-176	224
CTB	G_{M1}>G_{M2}>G_{M3}	-	-	-	-
PNA	Galβ1-3GalNAc	+++	+++	+	+
LFA	Neu5Ac	+	+	++	++
MAA	α2-3Neu5Ac	+	+	+	+
Anti-G_{M2}	G_{M2}	+	+	++	-
Anti-G_{M1}	G_{M1}	+++	+++	-	+++
Jacalin	α-D-Gal and Galβ1-3GalNAc	++	++	+	+
ConA	α-D-Man>α-D-Glc	-	-	-	-
VAA	β-D-Gal	+++	+++	+	+
DBA	Terminal α-D-GalNAc	-	-	-	-

-: No binding observed
+: Binding observed at lectin print concentration of 500 µg/mL (lectin) or 1:100 diltution (antibody).
++: Binding observed at lectin print concentration of 250 µg/mL (lectin) or 1:1000 diltution (antibody).
+++: Binding observed at lectin print concentration of 125 µg/mL (lectin) or 1:10000 diltution (antibody).

Table 2: Results of the lectin array of phenol purified and crudely prepared LOS from *C. jejuni* strains NCTC 11168, 81-176 and 224.

A

Strain	6	8	5\10	12	14	16	Type	Known Type
224	+	+	-	+	-	+	R	N/A
11168	+	+	+	+	+	+	C	C
81-176	+	+	-	+	-	-	A/B	B

B

Cluster														
A	1	2	3	4	5	6	7	8	9	10	11	12	13	
B	1	2	3	4	5	6	7	8	9	5	10	11	12	13
C	1	2	3	4	14	15	6	7	8	9	5\10	16	12	13
R	1	2	3	4	5	6	7	8	9	10	16	12	13	
M	1	2	51	3	7	8	9	10	11	12	13			

Table 3: LOS Biosynthesis cluster analysis. **A).** Cluster types can be differentiated based on the presence of ORFs within the genome. *C. jejuni* strains 11168 and 81-176 were known due to the availability of genome sequences and were confirmed by PCR to be class C and A/B respectively. Strain 224 was found to be class R. **B).** Biosynthesis clusters capable of ganalioside molecular mimicry through production of sialylated LOS.

LOS biosynthesis cluster typing.

LOS biosynthesis clusters were typed using a PCR methodology based upon the published gene content of the LOS gene clusters [28,29]. Typing was performed using the primers and at annealing temperatures presented in (Table 1). Reactions were performed using 10 ρmoles of forward and reverse primer, 200 µM dNTPs, using the Phusion DNA Polymerase system (Finnzymes) to a final volume of 50 µL.

Lectin array preparation

Lectins were purchased from EY Laboratories and Sigma-Aldrich, and anti-ganglioside antibodies were purchased from Matreya (Table 2). Four identical sub-arrays per slide were printed essentially as previously described [30,31] onto epoxy functionalised glass slides (SuperEpoxy II; ArrayIt) at three different lectin concentrations (500 µg/mL, 250 µg/mL and 125 µg/mL), and anti-ganglioside antibodies were printed at 1:100 to 1:10,000 dilutions across a 10-fold serial dilution. Printed slides were subsequently neutralised using ethanolamine as previously described [31]. Lectins and antibodies printed on our array and their published specificities are presented in Table 2.

Lectin array analysis of *C. jejuni* LOS and whole *C. jejuni*

Purified and crudely isolated LOS was labelled using the lipophillic dye BODIPY® TR methyl ester (BODIPY; Invitrogen). One mM BODIPY (6 µL) was added to 5 µg of LOS in 1x PBS containing 1mM $CaCl_2$ and 1mM $MgCl_2$ to a final volume of 30 µL and incubated at room temperature for 15 min prior to direct application to printed lectin arrays. Subarrays were separated using 25µL gene frames (Thermo Scientific). LOS was incubated on the arrays for 15 minutes, followed by 3 washes with 1x PBS containing 1mM $CaCl_2$ and 1mM $MgCl_2$. Controls for BODIPY and unlabelled LOS were also applied to the array and washed in the same way. Whole *C. jejuni* was labelled using BODIPY by the addition of 1 mM BODIPY to 10^8 CFU of *C. jejuni* in 1x PBS and incubated at 37°C for 30 mins. Application of *C. jejuni* to the arrays and washing was performed as previously described [30].s Image acquisition and data processing was performed using

the ProScanArray Microarray 4-Laser Scanner and the ProScanArray imaging software ScanArray Express from PerkinElmer as previously described [30]. Analysis was limited to presence or absence of binding to lectin spots across a 1:2 serial dilution rather then absolute binding levels. All positive binding spots were confirmed by visual inspection of the array and tested significantly above the background, which was confirmed by two-tailed unpaired T-test in Microsoft Excel. Lectin arrays were performed a minimum of twice per LOS sample.

Results

The LOS of *C. jejuni* strain NCTC 11168 is known to mimic the human ganglioside structure, G_{M1}, and was therefore chosen to evaluate the viability of screening both phenol purified and crudely prepared LOS samples using our discrete lectin array. Initial analysis of the two alternatively isolated LOS samples from *C. jejuni* NCTC 11168 was performed using traditional SDS-PAGE silver staining and lectin blotting analysis (Figure 1). Silver staining revealed no differences in electrophoretic mobility between the phenol purified and crudely isolated LOS preparations (Figure 1A). Lectin blotting using HRP-conjugated PNA and CTB also revealed no differences in binding ability between sample preparations, with as expected [6] significant binding of both lectins (Figure 1B and 1C respectively). Taken together these data show that both LOS preparations have similar glycoconjugate components, specifically LOS that mimics G_{M1} (as identified through PNA and CTB positive binding), and hence both LOS preparations provide an excellent tool for assessment of lectin array technology. Due to the fact that the *C. jejuni* LOS structure with molecular mimicry with human gangliosides been reported [6,32-34], lectins were selected to generate a tailored array that would most effectively identify the individual carbohydrate motifs of structures containing monosialylganglioside mimicry (Table 2). The lectins selected included those known to bind the G_{M1} mimic *C. jejuni* NCTC 11168 LOS (PNA and CTB) [6,34,35], and those assumed to bind based on the published structures of NCTC 11168 and 81-176 [6,32],

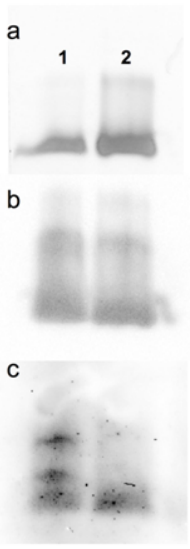

Figure 1: Silver-stained SDS-PAGE gel and Lectin blots of Crude and Phenol purified LOS preparations extracted from *C. jejuni* NCTC 11168. a). Silver stained LOS preparations. **b)**. CTB blotting result for LOS preparations. **c)**. PNA blotting result for LOS preparations. Lane 1 in each pane is Crude LOS preparation, while lane 2 is the phenol purified preparation.

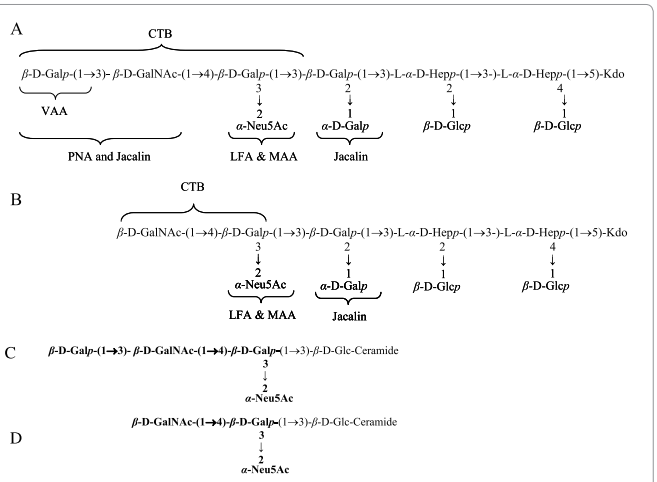

Figure 2: **Published structures of the LOS of *C. jejuni* NCTC 11168, 81-176 and human G_{M1} and G_{M2}. A).** *C. jejuni* NCTC 11168 [6] and the expected binding for lectins/antibodies present on the array. **B).** *C. jejuni* 81-176 [32] and the expected binding for lectins/antibodies present on the array. N.B. Expected lectin binding is predicted from specificities listed in Table 2. Lower specificity interactions are also possible. Presented structures are the published majority structure for each strain [6,32]. **C).** Structure of G_{M1} (bolded sugar residues are identical to published *C. jejuni* 11168 structure shown in **A**). **D).** Structure of G_{M2} (bolded sugar residues are identical to published 81-176 structure shown in **B**).

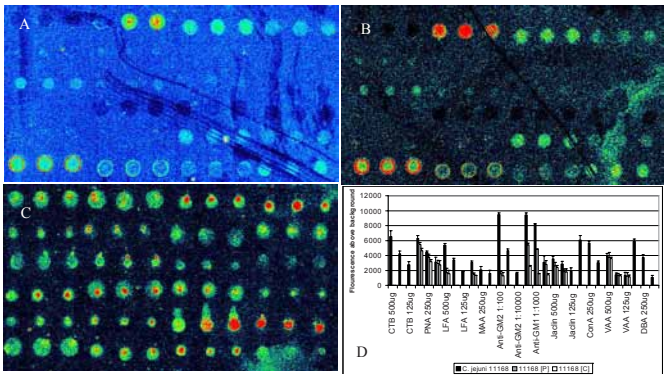

Figure 3: Analysis of live *C. jejuni* 11168 compared to purified LOS. A). Lectin array analysis of crude LOS extract prepared from *C. jejuni* 11168. **B).** Lectin array analysis of phenol purified LOS extract prepared from *C. jejuni* 11168. **C).** Lectin array analysis of live *C. jejuni* 11168. **D).** Graphical representation of the array analysis. Error bars equal one standard error of the mean. Any data not significantly different from background has been set to zero units. Lectins are listed in Table 2.

LFA (*Limax flavus* agglutinin), MAA (*Maackia amurensis* agglutinin), VAA (*Viscum album* agglutinin) and Jacalin (Jackfruit; *Artocarpus integrifolia*). Both DBA (*Dolichos biflorus* agglutinin) and ConA (*Canavalia ensiformis* agglutinin) were used as negative controls due to the absence of terminal GalNAc (recognised by DBA) and Man/GlcNAc (recognised by ConA) in the published structures [6,32]. In addition, two specific anti-ganglioside antibodies were also included, one anti-G_{M1} and one anti-G_{M2} (Figure 2).

Table 2 summarises the binding results obtained using our lectin array for both purified (11168 [P]) and crudely isolated (11168 [C]) LOS preparations. Even though binding of BODIPY labelled LOS to all lectins/antibodies with the exception of DBA, ConA and CTB was observed, some differences with respect to the level of binding was

observed (Table 2; Figure 3). That is, three lectins were found to bind both LOS preparations down to 125 µg/mL (lowest concentration printed), the β-Gal recognising lectins PNA and VAA, and the α-Gal recognising Jacalin (structures recognised shown in Figure 2). The lack of LOS binding to DBA and ConA was anticipated, however the inability of either LOS preparation to binding immobilised CTB was surprising, particularly given that lectin blot analysis using HRP-conjugated CTB showed significant binding (Figure 1B). Both anti-ganglioside antibodies were bound by both LOS preparations, however binding to anti-G_{M2} was only observed at the highest concentration printed, while anti-G_{M1} was bound down to the 1:1000 dilution (Table 2). This is not surprising given that the published *C. jejuni* LOS structure is known to be exclusively G_{M1} under specified growth conditions [35]. The low binding therefore observed to anti-G_{M2} may simply reflect some cross-specificity to the underlying G_{M2} structure within the G_{M1} mimic. In comparison, live *C. jejuni* 11168-O bound to all printed spots that contained protein including CTB (Figure 3C).

Further analysis was performed on LOS isolated using the crude method from other *C. jejuni* strains, one with a published LOS structure 81-176 (Structure shown in Figure 2) [32] and a strain without a published structure, *C. jejuni* 224. *C. jejuni* 224 was chosen for analysis as it has a LOS biosynthesis cluster (Class R; Table 3A) capable of producing molecular mimicry (Class A, B, C, R and M; Table 3B) [28,29].

Lectin array analysis confirmed *C. jejuni* 81-176 produces LOS with G_{M2} mimicry rather than G_{M1}. Binding was observed for the two highest concentrations of the Anti-G_{M2} antibody, but no binding was observed for G_{M1}. The LOS from 81-176 also had decreased binding for PNA, Jacalin and VAA when compared to NCTC 11168 further confirming the absence of the terminal galactose from the structure (Figure 2). Binding of the 81-176 LOS to LFA and MAA confirmed the presence of sialylation on the LOS.

Analysis of *C. jejuni* strain 224 revealed binding to the anti-G_{M1} antibody but other binding to structures lectins such as PNA, Jacalin and VAA were only equal in binding to those observed for 81-176 rather than NCTC 11168. The LOS isolated from *C. jejuni* 224 was confirmed to be sialylated due to positive binding by LFA and MAA. No binding was observed for the antiG_{M2} antibody.

Discussion

The use of lectin arrays to determine surface glycan structures on whole bacteria has been previously described [1; 8]; however, to our knowledge, our approach of analysing fluorescently labelled isolated LOS on lectin array is completely novel, and offers a powerful analytical technique. This is particular the case, because the use of whole bacteria on lectin arrays may not accurately reveal the nature of the glycosylation present on the cell. As previously stated, lectins and antibodies are glycoproteins [11,12], therefore carbohydrate recognising adhesins present on bacteria [13-17] may bind these structures, rather than immobilised lectins binding to bacterial surface glycoconjugates. Testing of whole *C. jejuni* found binding to all lectins printed including CTB which had failed to bind in all other experiments (Figure 3; Table 2). Our novel approach of utilising isolated LOS overcomes this potentially significant shortcoming. Using the *C. jejuni* 11168 LOS as a model our discrete lectin array identified all carbohydrate components (Table 2) as predicted from the published structure (Figure 2).

The success of our approach depended largely on the dye used to label the isolated LOS. The lipophilic BODIPY TR methyl ester specifically interacts with the hydrocarbon tail of the LOS. The lack of binding observed to both DBA and ConA on the array indirectly confirms this selective labelling, given that any contaminating glycoconjugates (eg. glycoproteins) co-isolated with the *C. jejuni* LOS that could have been labelled with BODIPY would be expected to bind DBA immobilised on our array. That is, terminal α-D-GalNAc structures are common in *N*-linked *C. jejuni* glycoproteins [36], and if labelled, would have been detected on our lectin array.

Analysis was also performed on two other strains of *C. jejuni* for LOS molecular mimicry, 81-176 and 224. *C. jejuni* 81-176 was chosen because, like NCTC 1168 [6], 81-176 has a published structure [32]. The results of the lectin array agreed with the known structure produced by 81-176, a G_{M2} mimicking LOS [32]. *C. jejuni* 81-176 is known to produce several other structures including G_{M3}, G_{D1b} and G_{D2}, however, these structures are present in smaller amounts than the G_{M2} mimicking structures [32]. Therefore it is unlikely these structures would be affecting the outcomes of the array analysis. A wider variety of anti-ganglioside antibodies may prove effective in identifying these less prevalent LOS structures.

C. jejuni 224 LOS analysed by lectin array indicated primarily G_{M1} mimicry from the antibody binding but was not 100% confirmed by the binding observed for the other lectins present on the array. Levels of binding for PNA, VAA and Jacalin were lower than those seen for the known G_{M1} mimic NCTC 11168 (Table 2). However, the LOS structure produced by *C. jejuni* 224 was sialylated providing further evidence for ganglioside mimicry. A previous study showed that the LOS from *C. jejuni* 224 was of the same size by electrophoresis as NCTC 11168 and bound strongly by CTB [35]. This result together with the lectin array result implicates strongly that the LOS is a G_{M1} mimic.

Although our lectin array data correlates well with the known *C. jejuni* 11168 and 81-176 LOS structures, a discrepancy between observed and expected binding results was noted. Specifically, no binding was observed to CTB immobilised on the lectin array, even though lectin blotting using CTB (Figure 1) showed a strong positive signal. This was a predictable outcome since CTB exists as a pentameric structure [37]. The complex pentameric structure of CTB may therefore be disrupted or constrained when covalently attached to the array through an epoxside-linkage. CTB is likely to be unsuitable for use on lectin arrays and its use restricted to lectin blotting analyses.

We also investigated the suitability of two different LOS preparations (phenol-purified and crudely isolated LOS) for analysis on our lectin array. Apart from a slight increase in non-specific background binding of the crude LOS preparation, compared to the phenol purified LOS (data not shown), no significant difference was observed using either LOS preparations. This suggests that a simple heat lysis and proteinase K digestion of *C. jejuni* or other bacteria is sufficient to allow rapid and sensitive screening using lectin arrays particularly of strains expressing ganglioside mimicry. The simple methodology reported here can, therefore, be used to rapidly evaluate whether clinical isolates have the potential to produce adverse autoimmune reactions as post infection sequelae, similar to those attributed to *C. jejuni* (GBS or MFS). Knowing whether clinically important bacterial strains are capable of inducing severe autoimmune responses may aid in prevention and/or early diagnosis of these debilitating post infection conditions.

The method reported here is also applicable, through minor modifications to the lectins/antibody specificities printed on the array, to the screen glycolipids from almost any other species of bacteria for almost any terminal glycoconjugate.

References

1. Hsu KL, Pilobello KT, Mahal LK (2006) Analyzing the dynamic bacterial glycome with a lectin microarray approach. Nat Chem Biol 2: 153-157.
2. Gilbert M, Brisson JR, Karwaski MF, Michniewicz J, Cunningham AM, et al. (2000) Biosynthesis of ganglioside mimics in Campylobacter jejuni OH4384. Identification of the glycosyltransferase genes, enzymatic synthesis of model compounds, and characterization of nanomole amounts by 600-mhz (1)h and (13)c NMR analysis. J Biol Chem 275: 3896-3906.
3. Gilbert M, Parker CT, Moran AP (2008) Campylobacter jejuni lipooligosaccharides: structures and biosynthesis ASM Press Washington, DC.
4. Moran AP, Annuk H, Prendergast MM (2005) Antibodies induced by ganglioside-mimicking Campylobacter jejuni lipooligosaccharides recognise epitopes at the nodes of Ranvier. J Neuroimmunol 165: 179-185.
5. Peak IR, Grice ID, Faglin I, Klipic Z, Collins PM, et al. (2007) Towards understanding the functional role of the glycosyltransferases involved in the biosynthesis of Moraxella catarrhalis lipooligosaccharide. Febs J 274: 2024-2037.
6. St Michael F, Szymanski CM, Li J, Chan KH, Khieu NH, et al. (2002) The structures of the lipooligosaccharide and capsule polysaccharide of Campylobacter jejuni genome sequenced strain NCTC 11168. Eur J Biochem. 269: 5119-5136.
7. Fogg GC, Yang LY, Wang E, Blaser MJ (1990) Surface array proteins of Campylobacter fetus block lectin-mediated binding to type A lipopolysaccharide. Infect Immun 58: 2738-2744.
8. Hsu KL, Mahal LK (2006) A lectin microarray approach for the rapid analysis of bacterial glycans. Nat Protoc 1: 543-549.
9. Tao SC, Li Y, Zhou J, Qian J, Schnaar RL, et al. (2008) Lectin microarrays identify cell-specific and functionally significant cell surface glycan markers. Glycobiology 18: 761-769.
10. Tateno H, Uchiyama N, Kuno A, Togayachi A, Sato T, et al. (2007) A novel strategy for mammalian cell surface glycome profiling using lectin microarray. Glycobiology 17: 1138-1146.
11. Ashford DA, Dwek RA, Rademacher TW, Lis H, Sharon N (1991) The glycosylation of glycoprotein lectins. Intra- and inter-genus variation in N-linked oligosaccharide expression. Carbohydr Res 213: 215-227.
12. Debray H, Wieruszeski JM, Strecker G, Franz H (1992) Structural analysis of the carbohydrate chains isolated from mistletoe (Viscum album) lectin I. Carbohydr Res 236: 135-143.
13. Aspholm-Hurtig M, Dailide G, Lahmann M, Kalia A, Ilver D, et al. (2004) Functional adaptation of BabA, the H. pylori ABO blood group antigen binding adhesin. Science 305: 519-522.
14. Gilboa-Garber N, Sudakevitz D (1999) The hemagglutinating activities of Pseudomonas aeruginosa lectins PA-IL and PA-IIL exhibit opposite temperature profiles due to different receptor types. FEMS Immunol Med Microbiol 25: 365-369.
15. Lehmann F, Tiralongo E, Tiralongo J (2006) Sialic acid-specific lectins: occurrence, specificity and function. Cell Mol Life Sci 63: 1331-1354.
16. Thomas WE, Nilsson LM, Forero M, Sokurenko EV, Vogel V (2004) Shear-dependent 'stick-and-roll' adhesion of type 1 fimbriated Escherichia coli. Mol Microbiol 53: 1545-1557.
17. Thomas WE, Trintchina E, Forero M, Vogel V, Sokurenko EV (2002) Bacterial adhesion to target cells enhanced by shear force. Cell 109: 913-923.
18. Szymanski CM, Logan SM, Linton D, Wren BW (2003) Campylobacter--a tale of two protein glycosylation systems. Trends Microbiol 11: 233-238.
19. Nachamkin I, Allos BM, Ho T (1998) Campylobacter species and Guillain-Barre syndrome. Clin Microbiol Rev 11: 555-567.
20. Prendergast MM, Moran AP (2000) Lipopolysaccharides in the development of the Guillain-Barre syndrome and Miller Fisher syndrome forms of acute inflammatory peripheral neuropathies. J Endotoxin Res 6: 341-359.
21. Yuki N, Susuki K, Koga M, Nishimoto Y, Odaka M, et al. (2004) Carbohydrate mimicry between human ganglioside GM1 and Campylobacter jejuni lipooligosaccharide causes Guillain-Barre syndrome. Proc Natl Acad Sci U S A 101: 11404-11409.
22. Gaynor EC, Cawthraw S, Manning G, MacKichan JK, Falkow S, et al. (2004) The genome-sequenced variant of Campylobacter jejuni NCTC 11168 and the original clonal clinical isolate differ markedly in colonization, gene expression, and virulence-associated phenotypes. J Bacteriol 186: 503-517.
23. Hitchcock PJ, Brown TM (1983) Morphological heterogeneity among Salmonella lipopolysaccharide chemotypes in silver-stained polyacrylamide gels J Bacteriol. 154: 269-277.
24. Westphal O, Luderitz O, Bister O (1952) Uber die Extraktion von Bakterien mit Phenol/Wasser Naturforsch. 7: 148-155.
25. Moran AP, Helander IM, Kosunen TU (1992) Compositional analysis of Helicobacter pylori rough-form lipopolysaccharides J Bacteriol. 174: 1370-1377.
26. H Schagger (2006) Tricine-SDS-PAGE Nat Protoc. 1: 16-22.
27. Tsai CM, Frasch CE (1982) A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels Anal Biochem. 119: 115-119.
28. Parker CT, Gilbert M, Yuki N, Endtz HP, Mandrell RE (2008) Characterization of lipooligosaccharide-biosynthetic loci of Campylobacter jejuni reveals new lipooligosaccharide classes: evidence of mosaic organizations J Bacteriol. 190: 5681-5689.
29. Parker CT, Horn ST, Gilbert M, Miller WG, Woodward DL, et al. (2005) Comparison of Campylobacter jejuni lipooligosaccharide biosynthesis loci from a variety of sources J Clin Microbiol. 43: 2771-2781.
30. Day CJ, Tiralongo J, Hartnell RD, Logue CA, Wilson JC, et.al (2009) Differential carbohydrate recognition by Campylobacter jejuni strain 11168: influences of temperature and growth conditions PLoS One 4: e4927.
31. Hartley-Tassell LE, Shewell LK, Day CJ, Wilson JC, Sandhu R, et al. (2010) Identification and characterization of the aspartate chemosensory receptor of Campylobacter jejuni Mol Microbiol. 75: 710-730.
32. Guerry P, Szymanski CM, Prendergast MM, Hickey TE, Ewing CP, et al. (2002) Phase variation of Campylobacter jejuni 81-176 lipooligosaccharide affects ganglioside mimicry and invasiveness in vitro Infect Immun. 70: 787-793.
33. Karlyshev AV, Ketley JM, Wren BW (2005) The Campylobacter jejuni glycome. FEMS Microbiol Rev 29: 377-390.
34. Linton D, Gilbert M, Hitchen PG, Dell A, Morris HR, et al. (2000) Phase variation of a beta-1,3 galactosyltransferase involved in generation of the ganglioside GM1-like lipo-oligosaccharide of Campylobacter jejuni Mol Microbiol 37: 501-514.
35. Semchenko EA, Day CJ, Wilson JC, Grice ID, Moran AP, et al. (2010) Temperature-dependent phenotypic variation of Campylobacter jejuni lipooligosaccharides BMC Microbiol. 10: 305.
36. Young NM, Brisson JR, Kelly J, Watson DC, Tessier L, et al. (2002) Structure of the N-linked glycan present on multiple glycoproteins in the Gram-negative bacterium, Campylobacter jejuni J Biol Chem. 277: 42530-42539.
37. Zhang RG, Westbrook ML, Westbrook EM, Scott DL, Otwinowski Z, et al. (1995) The 2.4 A crystal structure of cholera toxin B subunit pentamer: choleragenoid. J Mol Biol 251: 550-562.