Appl Microbiol Biotechnol
DOI 10.1007/s00253-006-0723-9

METHODS

Fabrication and application of carbohydrate microarray for analyzing human serum antibody–carbohydrate interaction

Gang-Liang Huang · Xin-Ya Mei · Han-Xing Zhang · Peng-George Wang

Received: 23 August 2006 / Revised: 11 October 2006 / Accepted: 14 October 2006

Abstract We introduced a strategy for preparing a carbohydrate microarray and demonstrated its utility for characterizing carbohydrate binding and activities. We isolated the lipopolysaccharide (LPS) components from different bacteria and explored the possibility of immobilizing these glycoconjugates on a high-binding polystyrene plate. Carbohydrate-specific combination was examined by observing the binding of the blood group B analogic LPS *O*-polysaccharide from *Escherichia coli* on the high-binding polystyrene plate and anti-B from a broad spectra antibody of human blood serum. Strong binding of antibodies was screened, as it was evident that relative response value is two times higher than control. The hybridization results indicated that this method is a reliable technique for the detection of human intestinal bacteria and is expected to be applied in diagnostics and seroepidemiology.

Introduction

The ABO blood group specificity is determined by the nature and linkage of monosaccharides at the ends of the carbohydrate chains (Fig. 1). Antibodies to ABO antigen structures are among the most predominant naturally occurring antibodies, and they were found to recognize carbohydrates rather than proteins. Some strains of enteric bacteria, such as *Escherichia coli* O86, O127, and O128, have been the natural antigens that stimulate the generation of human natural antibodies. Springer (1956) reported a high blood group B activity of *E. coli* O86 in its lipopolysaccharide (LPS) O-chain. The research was carried out to explore the immunogenecity of *E. coli* O86 strains with human serum using carbohydrate-based microarray system. Besides *E. coli* O86, two closely related strains *E. coli* O127 and O128 were studied, since they were reported to possess anti-blood group O activity (Salles et al. 1989; Springer and Horton 1969). The primary structures of LPS O-polysaccharide from these three strains have been elucidated chemically, which showed different structures in antigen repeat units in Fig. 1.

Carbohydrate microarray is a state-of-art technology and it has demonstrated the feasibility to print carbohydrate-containing molecules onto nitrocellulose-coated glass slides (Wang et al. 2002). Microarray also presents ligands in a multivalent manner, and several different types of carbohydrate microarrays were designed utilizing both naturally derived and synthetic glycans on media ranging from multi-well plates to modified glass slides (Bryan et al. 2004; Fukui et al. 2002; Adams et al. 2003, 2004; Houseman and Mrksich 2002). Recently, Disney and Seeberger (2004) reported the use of specific binding carbohydrate microarrays to detect fluorescently labeled bacteria in complex biological samples. Thirumalapura et al. (2005) evaluated the use of LPS microarrays printed on nitrocellulose-coated slides as a platform for the detection of anti-LPS antibodies.

In this study, carbohydrate microarray was prepared by a novel procedure that allows the immobilization of LPS components to a high-binding polystyrene plate without using the expensive nitrocellulose microarray. LPS purified from *E. coli* O86, O127, and O128 by a sample method preserved the accessibility of epitopes for antibody binding. Attempts have been made to exploit the

G.-L. Huang (✉) · X.-Y. Mei · H.-X. Zhang · P.-G. Wang
State Key Laboratory of Microbial Technology (SKLMT),
Shandong University,
Jinan, Shandong 250100, People's Republic of China
e-mail: hgl226@126.com

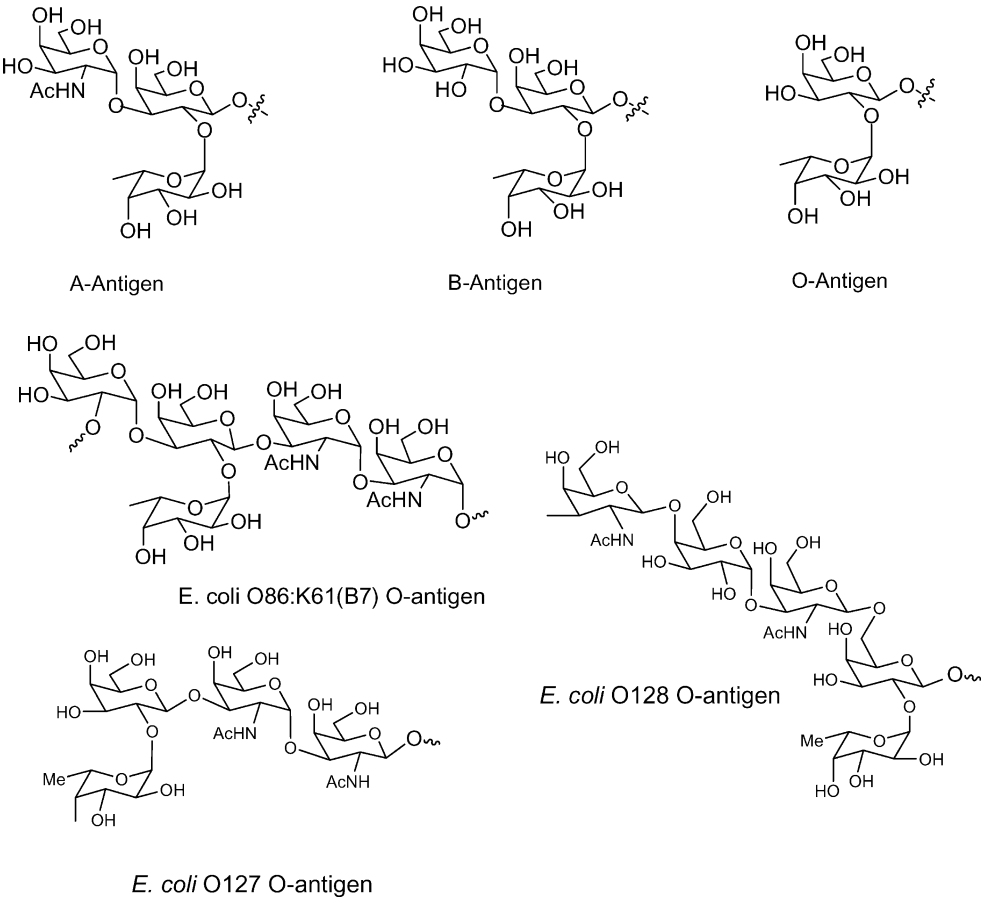

Fig. 1 Proposed representative carbohydrate chains of human blood group A, B, O and different carbohydrate structures of *E. coli* O-antigen repeat units

saccharide moieties of human serums for binding through carbohydrate affinity (Ailus and Palosuo 1995), describing the bacteria with different carbohydrate structure and profiling the interaction roles of carbohydrate structure and microorganism infection.

Materials and methods

Preparation of bacterial whole cell lysate antigens and LPS antigens

Different bacterial LPS compounds were purified from *E. coli* O86, O127, and O128. The microarrays were also designed to contain *E. coli* BL21 and DH5α as negative control.

All the five virulent bacteria grown separately in Luria–Bertani medium at 37°C were harvested at 20 h and centrifuged at 10,000×*g* for 10 min. The cells were washed twice in 0.01 mM phosphate buffer saline (PBS), pH 7.4, and resuspended. The cells killed by phenol were sonicated at 30 Hz for 5 min and centrifuged at 10,000×*g* for 10 min. The supernatant was collected and condensed and used as whole-cell lysate antigens. The LPS antigens were prepared according to the method of Westphal et al. (1983).

Serum samples and second antibody materials

Human anti-B sera of group A, human sera of group AB, and some erythrocytes B were supplied by Shandong Provincial Hospital, China. The sera of type A were arranged on the basis of their reactivity against blood group B antigen, and the group AB was used as control. Anti-human IgG and IgM were purchased from Sigma Chemical.

Hemagglutination inhibition assay

To investigate whether purified components from *E. coli* preserved antigenic determinants have carbohydrate activities, polysaccharide preparations of whole-cell lysate antigens were studied using hemagglutination inhibition binding assay Fig. 2. Isolated LPS was used as positive control. The red blood cell B responds to anti-B sera by carbohydrate determinant. LPS purified from various *E. coli* with different sugar structure was used to bind with anti-B sera, by competition with the red blood cell B.

All reagents were employed in a volume of 0.1 ml. In each tube, 0.1 ml of bacterial whole lysates and LPS was used. The suspensions were shaken and incubated with anti-B serum for 2 h at room temperature (22–26°C). The erythrocytes B were then added, and the test was read

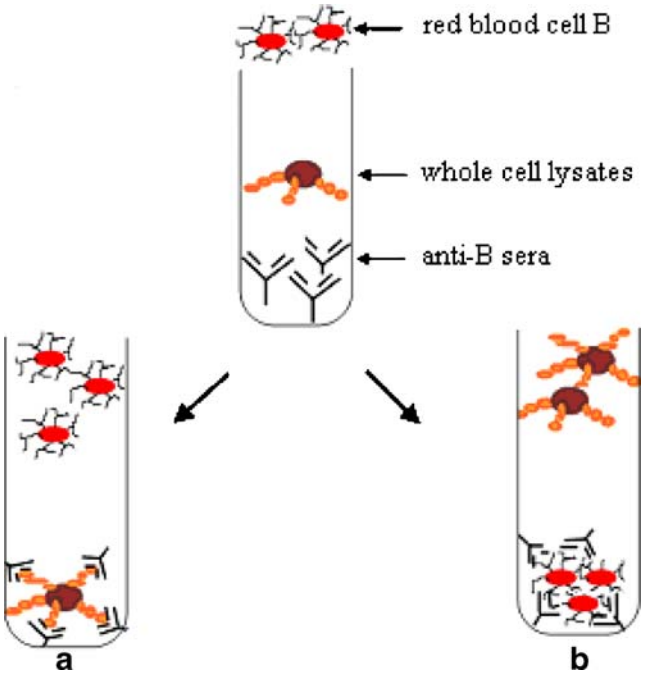

Fig. 2 Sketch map of the principle of hemagglutination inhibition assay. The reaction of whole cell lysates with anti-B sera will inhibit the reaction of red cell B, resulting in colorless deposits on the bottom of the tube (**a**). Otherwise, the red cells will react with the anti-B sera and will thus produce red deposits on the bottom of the tube (**b**), which suggests that the whole cell lysates purified from *E. coli* have no carbohydrate activities

microscopically after a further incubation of 1 to 2 h at room temperature.

Printing bacterial antigen microarray and antibody-binding experiment

The components of microbial antigens treated above were printed at different concentrations on the Costar brand 96-well half area high-binding polystyrene plate (Huang et al. 2005, 2006a,b), A high-precision robot (Pixsys 5500 Cartesian Technologies) was utilized to spot different antigen preparations. For LPS immobilization studies, carbohydrate antigens were dissolved in PBS, then printed at an initial concentration of 9 mg ml^{-1} and diluted by a 1:2 series titration in 0.01 M PBS, pH 7.4. Microarrays consisted of a 5×10 matrix that included five microbial antigens that were printed in two replicates, using *E. coli* BL21 and DH5α as negative control. The first two points in line 3 are used as blank (Fig. 3a). Before use, the printed microarrays were blocked with 3% bovine serum albumin diluted in PBS-T 60. Then the chip was incubated at 37°C for 30 min with human sera anti-B diluted 1:100. The human anti-bacterial antibodies were dectected by using biotinylated anti-human IgM and visualized by a cy3-streptavidin.

Additionally, another antibody-binding experiment was carried out using various blood types. Whole cell lysates of *E. coli* O86, O127, and O128 antigen were arrayed on plates in two replicates at concentrations of 4.5, 2.25, and 1.12 mg ml^{-1}, respectively (Fig. 4a). They were incubated with combined human serum specimens at a concentration equivalent to 1:100 dilution for each specimen. The human IgM captured by microarray was visualized by a biotinylated anti-human IgM and cy3-streptavidin. The human IgG was detected using a biotinylated anti-human IgG, and the color was developed by using cy3-streptavidin.

The stained microarrays were scanned with a Biochip Scanning System (Scanarray 4000). The printed bacterial microarrays were air-dried and stored at room temperature without desiccant before application.

Results

Bacterial antigen activity assay of the hemagglutination inhibition test

The ability of various *E. coli* with different sugar structures to bind with anti-B by competition with red blood cell B was shown in Table 1. The results showed high antigenic similarities among the bacterial whole cell lysates of *E. coli* O86 and O127, which have somatic B antigens that can adsorb anti-B in blood A sera, and the red blood cell B was on the surface of the liquid (−). Therefore, *E. coli* O86 and O127 were the best inhibitors for the hemagglutination of anti-B competed with red blood cell B. However, few or no similarities were observed in components of *E. coli* O128, BL21, and DH5α, and there was little dilution of the serum being adsorbed. The anti-B acted strongly with red blood cell B; therefore, the cells were hemagglutinated and deposited on the bottom of the tube (+).

Immobilization of LPS components isolated from different bacteria on microarray

The avidity of *E. coli* LPS binding with human blood anti-B are summarized in Fig. 3a, and the interaction profiles are shown in Fig. 3b. The results showed a corresponding fluorescence intensity decrease correlating with the dilution. Spots containing antigens above 225~112.5 μg ml^{-1} showed optimal response where specific IgM levels of *E. coli* O86 were measured with immunostaining microarrays. Among the five kinds of *E. coli* LPS tested for binding, *E. coli* O86 reacted strongly with human blood anti-B, *E. coli* O127 gave low absorbance values, and the other three bacteria reacted lowly with anti-B.

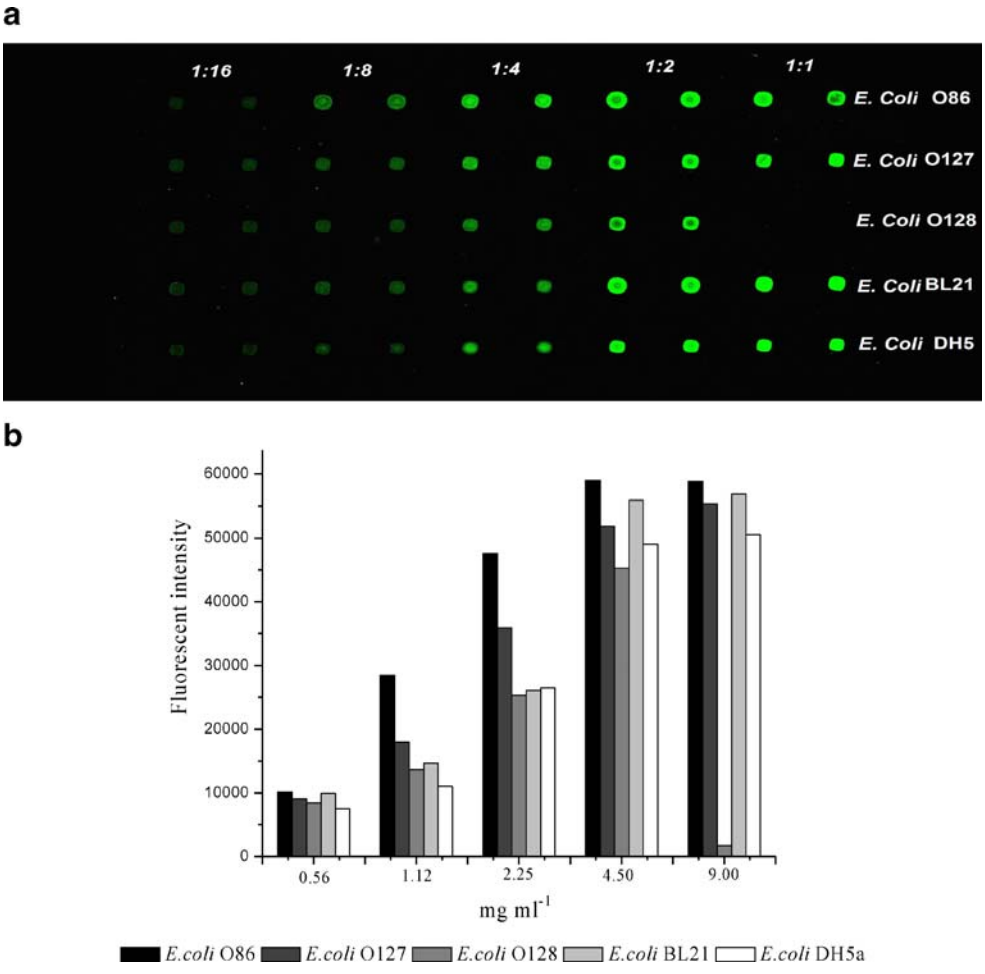

Fig. 3 **a** Image of microarray plate incubated with antisera from human serum sample blood. **b** Quantitative illustration of antibodies captured specifically by a carbohydrate microarray

Microarray to detect the specificity of antibody from serum of various blood types

To further demonstrate the restricted specificity of this carbohydrate antigen, the microarray was applied to detect human serum antibodies. The presence of anti-B and anti-Gal reactivity within the glycoconjugate of *E. coli* O86 were also demonstrated using microarray printed, as shown in Fig. 4a. This was shown by microarrary analysis in Fig. 4b.

E. coli O86 was positively stained in microarray I, captured anti-B in human serum of IgM isotype. The fluorescent intensity was more than two times than others for anti-B antibodies of blood type A individuals containing anti-Gal activity. *E. coli* O86 antigen was positively stained in microarray II and IV, captured anti-Gal antibodies of IgG isotype. Its fluorescent intensity was 1.5 times higher than others. These results indicated that the antibody of IgG serum has a broader spectra of specificity than antibody IgM, since these virulent strains of *E. coli* were most frequently found in the repertoire of human myeloma to bore antibodies in IgG class (Greenwell 1997). A low hemagglutination adsorption of B-like antigens was observed with anti-B and anti-Gal from AB serum in microarray III, for there is no anti-B existing in AB serum. The concentration of anti-Gal in IgM class is low.

Discussion

Bacterial antigen activity assay of the hemagglutination inhibition test

The results showed that not only was the adsorption of anti-B more efficient with LPS from *E. coli* O86 and O127 than with red blood cells, but also the antibody was better identified by the carbohydrate antigen sharing in the components of bacterial pathogens such as whole cell lysates. The antibody reacted with group B cells and B antigen analogues but not with other related oligosaccharides (Springer and Horton 1969). The LPS components isolated from different bacterial pathogens showed high antigenic activity.

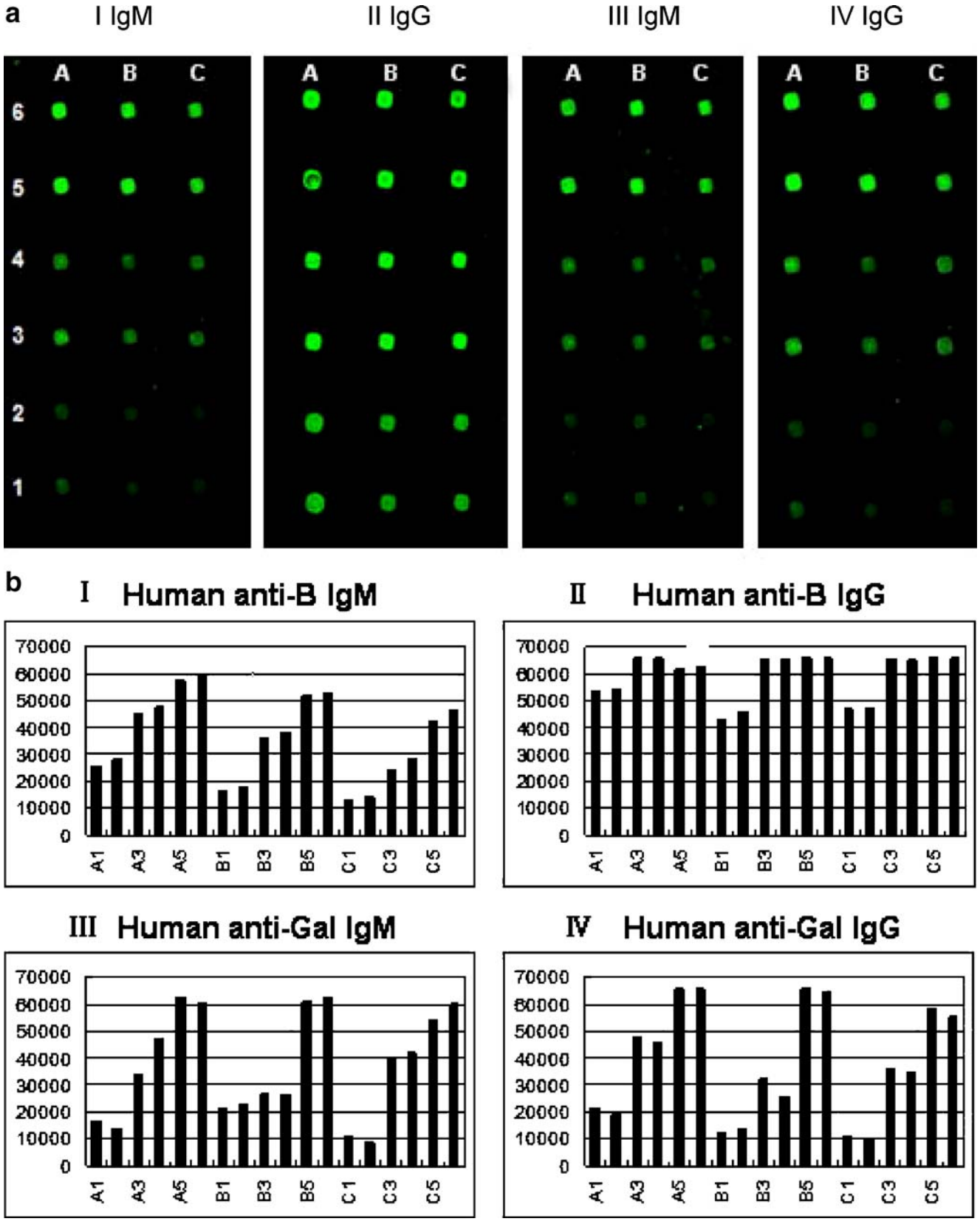

Fig. 4 A carbohydrate microarray characterization of human antibodies. **a** Images of antibody-stained LPS microarrays. **b** Quantitative illustration of antibodies captured specifically by a carbohydrate microarray. Fluorescent scans of antigen arrays incubated with samples from human blood A serum (*I*, *II*) and human blood AB serum (*III*, *IV*), which differ according to their reactivities with human anti-carbohydrate antibodies identified by microarray assay

Table 1 Hemagglutination inhibition test of different bacteria in the presence of B antigen and hemagglutination inhibited antiserum

	E. coli				
	O86	O127	O128	BL21	DH5α
Whole cell lysates	−	−	+	+	+
LPS	−	−	+	+	+

A negative sign denotes high inhibition (strong positive), while a positive sign denotes no inhibition.

Immobilization of LPS components isolated from different bacteria on microarray

By analyzing the fluorescent intensities retained on the plates after detecting the presence of specific antibodies anti-B, we demonstrated that preparations of whole cell lysate antigens from *E. coli* were stably immobilized on the high-binding polystyrene plate. These results confirmed the presence of the immobilized carbohydrate on the plates. This component is well-suited for immobilization because it is rapid, quantitative, and selective.

The structure of the five-carbohydrate antigens is similar to each other and special identification was achieved by carbohydrate microarray. Noticeably, human blood B trisaccharide epitope on the red blood cell consists of α-L-Fuc (1→2)[α-D-Gal(1→3)]β-D-Gal-OMe structure (Andersson et al. 1989), which is similar to the *E. coli* O86 O-antigen Galα1→3(Fuc1→2)Gal (Galili et al. 1987). The microarrays assay showed that this structure was possibly responsible for the binding of LPS of *E. coli* O86 to the human anti-B. Furthermore, anti-B reacted strongly with complex carbohydrates with Galα1→3(Fuc1→2)Gal clusters of *E. coli* O86. The response value was the best and about 1.8 times of Fuc1→2Gal from *E. coli* O127, and two times of *E. coli* O128. Without this carbohydrate structure, the response value of *E. coli* BL21 and DH5α are very low. Comparison of *E. coli* O86 and O127 O-polysaccharide structure revealed that they have the same sugar polymer backbone, the only difference being in the sugar side chain. In *E. coli* O86 O-polysaccharide, one more galactoside is linked to α-D-Gal residue in its sugar backbone through the α-1, 3 glycosidic linkage.

Microarray to detect the specificity of antibody from serum of various blood types

Similar to the production of anti-blood group antibodies by bacterial antigens, bacteria within normal intestinal flora also provide constant antigenic stimulation for the synthesis of anti-Gal, which abundantly exists in human serum. Anti-Gal is a polyclonal antibody present in every individual and constitutes as much as 1% of the circulating IgG. It is defined as an antibody that interacts with the Galα1→3Gal epitope (Seidl et al. 1997). Therefore, anti-B antibodies of blood type A individuals contain anti-Gal activity. However, the spectrum of anti-Gal specificity is dependent on the individual's blood type. Thus, B and AB type individuals produce anti-Gal antibodies that only bind to Galα1→3Gal epitope; whereas A or O type individuals who lack the B epitope on their red cells have clones of anti-Gal that bind to both Galα1→3Gal and the B antigen.

A simple, rapid, and efficient method for the noncovalent binding of carbohydrate to high-binding polystyrene plate was developed. All these results clearly indicated that the carbohydrate microarrays prepared from whole cell lysates of bacteria showed immunological activities. Our findings demonstrate that good analytical and clinical data can be obtained with microarrays. This method may have potentially important advantages in convenience and cost compared with nitrocellulose carbohydrate chip. Moreover, development of a microarray-based bacterial identification system, directly from the biological sample and without any enzymatic target amplification (gene-chip used), would be mostly welcomed. The method-prepared carbohydrate antigen may have practical applications in carbohydrate material for high-throughout detection. Microarray-based bacterial identification has the potential to improve and could be used in the diagnosis of infectious diseases and pathologic conditions, such as *E. coli* infection, and autoimmune diseases in the near future.

References

Adams EW, Ueberfeld J, Ratner DM, O'Keefe BR, Walt DR, Seeberger PH (2003) Encoded fiber-optic microsphere arrays for probing protein-carbohydrate interactions. Angew Chem Int Ed Engl 42:5317–5320

Adams EW, Ratner DM, Bokesch HR, McMahon JB, O'Keefe BR, Seeberger PH (2004) Oligosaccharide and glycoprotein microarrays as tools in HIV glycobiology; glycan-dependent gp120/protein interactions. Chem Biol 11:875–881

Ailus K, Palosuo T (1995) IgM class autoantibodies in human cord serum. J Reprod Immunol 29:61–67

Andersson M, Carlin N, Leontein K, Lindquist U, Slettengren K (1989) Structural studies of the O-antigenic polysaccharide of Escherichia coli O86, which possesses blood-group B activity. Carbohydr Res 185:211–223

Bryan MC, Fazio F, Lee HK, Huang CY, Chang A, Best MD, Calarese DA, Blixt O, Paulson JC, Burton D, Wilson IA, Wong CH (2004) Covalent display of oligosaccharide arrays in microtiter plates. J Am Chem Soc 126:8640–8641

Disney MD, Seeberger PH (2004) The use of carbohydrate microarrays to study carbohydrate–cell interactions and to detect pathogens. Chem Biol 11:1701–1707

Fukui S, Feizi T, Galustian C, Lawson AM, Chai W (2002) Oligosaccharide microarrays for high-throughput detection and specificity assignments of carbohydrate–protein interactions. Nat Biotechnol 20:1011–1017

Galili U, Buehler J, Shohet SB, Macher BA (1987) The human natural anti-Gal IgG. III. The subtlety of immune tolerance in man as

demonstrated by crossreactivity between natural anti-Gal and anti-B antibodies. J Exp Med 165:693–704

Greenwell P (1997) Blood group antigens: molecules seeking a function? Glycoconj J 14:159–173

Houseman BT, Mrksich M (2002) Carbohydrate arrays for the evaluation of protein binding and enzymatic modification. Chem Biol 9:443–454

Huang GL, Liu TC, Liu MX, Mei XY (2005) The application of quantum dots as fluorescent label to glycoarray. Anal Biochem 340:52–56

Huang GL, Huang HL, Zhang HC, Wang PG (2006a) Structure–function relations of carbohydrates by neoglycolipid arrays. Appl Biochem Biotechnol 133:211–215

Huang GL, Zhang HC, Wang PG (2006b) Fabrication and application of neoglycolipid arrays in a microtiter plate. Bioorg Med Chem Lett 16:2031–2033

Salles MF, Mandine E, Zalisz R, Guenounou M, Smets P (1989) Protective effects of murine monoclonal antibodies in experimental septicemia: E. coli antibodies protect against different serotypes of *E. coli*. J Infect Dis 159:641–647

Seidl KJ, MacKenzie JD, Wang D, Kantor AB, Kabat EA, Herzenberg LA, Herzenberg LA (1997) Recurrent identical rearrangement and repeated expression of identical heavy and light chains in single anti-phosphatidylcholine B cells. Ann N Y Acad Sci 815:484–488

Springer GF (1956) Inhibition of blood-group agglutinins by substances occurring in plants. J Immunol 76:399–407

Springer GF, and Horton RE (1969) Blood group isoantibody stimulation in man by feeding blood group-active bacteria. J Clin Invest 48:1280–1291

Thirumalapura NR, Morton RJ, Ramachandran A, Malayer JR (2005) Lipopolysaccharide microarrays for the detection of antibodies. J Immunol Methods 298:73–81

Wang D, Liu S, Trummer BJ, Deng C, Wang A (2002) Carbohydrate microarrays for the recognition of cross-reactive molecular markers of microbes and host cells. Nat Biotechnol 20: 275–281

Westphal O, Jann K, Himmelspach K (1983) Chemistry and immunochemistry of bacterial lipopolysaccharides as cell wall antigens and endotoxins. Prog Allergy 33:9–39