

High-Throughput Carbohydrate Microarray Technology

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Summary

One of our long-term interests is to explore the immunogenic sugar moieties that are important for “self-” and “nonself” discrimination and host immune responses. We have established a high-throughput platform of carbohydrate microarrays to facilitate these investigations. Using this technology, carbohydrate-containing macromolecules of distinct structural configurations, including polysaccharides, natural glycoconjugates, and mono- and oligosaccharides coupled to lipid, polyacrylamide, and protein carriers, have been tested for microarray construction without further chemical modification. Here, we discuss issues related to the establishment of this technology and areas that are highly promising for its application. We also provide an example to illustrate that the carbohydrate microarray is a discovery tool; it is particularly useful for identifying immunological sugar moieties, including differentially expressed complex carbohydrates of cancer cells and stem cells as well as sugar signatures of previously unrecognized microbial pathogens.

Key Words: Antibodies; antigens; carbohydrates; glycans; glycoconjugates; microarrays; microspotting; nitrocellulose; polysaccharides; severe acute respiratory syndrome-associated coronavirus; SARS-CoV.

1. Introduction

Our group has focused on development of a carbohydrate-based microarray technology to facilitate investigation of carbohydrate-mediated molecular recognition and anticarbohydrate immune responses (1–3). Like nucleic acids and proteins, carbohydrates are another class of the essential biological molecules. Because of their unique physicochemical properties, carbohydrates are capable of generating structural diversity, and so they are prominent in display on the surfaces of cell membranes or on the exposed regions of macromolecules (4–6). As a result, carbohydrate moieties are suitable for storing biological signals in the forms that are identifiable by other biological systems. In this chapter, we discuss 1) our theoretical consideration for developing high-throughput carbohydrate microarrays, 2) a unique approach we took to establish carbohydrate microarrays, 3) a practical carbohydrate microarray platform that is currently in use by our laboratory, and 4) an example that illustrates a highly promising area for carbohydrate microarray technology to explore.

2. Theoretical Considerations in Developing Carbohydrate Microarrays

The Genome Project has led to the discovery that only approx 30,000 genes in the human genome must account for all the complexity of the human organism. This discovery raised an important question about the roles of protein processing and structural modification in modulating the biological activities of proteins and cellular functions. In higher eukaryotic species, most secretory and membrane-bound proteins are decorated with sugar moieties, which is achieved by a critically important posttranslational protein modification, called glycosylation. In many physiological and pathophysiological conditions, expression of cellular glycans, in the form of either glycoproteins or glycolipids, is differentially regulated. There are documented examples that show that cell display of precise complex carbohydrates is characteristically associated with the stages or steps of embryonic development, cell differentiation, and transformation of normal cell to abnormally differentiated tumor or cancer cells (7–10). Sugar moieties are also abundantly expressed on the outer surfaces of the mass majority of viral, bacterial, protozoan, and fungal pathogens. Many sugar structures are pathogen specific, which makes them important molecular targets for pathogen recognition, diagnosis of infectious diseases, and vaccine development (4,11–15).

In spite of the biological magnitude of carbohydrate molecules, the characterization of carbohydrate structures and the exposition of their function have lagged compared with other major classes of biological molecules, such as nucleic acids and proteins. For example, whereas the microarray-based high-throughput technologies for nucleic acids (16,17) and proteins (18,19) were developed years ago, the first carbohydrate microarray research was published in 2002 (1,20–24).

Our endeavor has focused on establishment of a high-throughput carbohydrate microarray platform that is technically equivalent to the state-of-the-art technologies of the cDNA microarray. Carbohydrates are strikingly different from nucleic acids in structure, physicochemical properties, and cellular function. Thus, the fundamental principles that are at the basis of establishment of a carbohydrate-based assay differ from the basic principles of the nucleic acid-based assays, such as the cDNA microarray and oligonucleotide biochips. For the nucleic acid-based biochips, the detection specificity is determined by the A::T and C::G base pairing, and there is no need to preserve the three-dimensional (3D) structures of the nucleic acid molecules. By contrast, carbohydrate microarrays require preservation of the 3D conformations and topological configurations of sugar moieties on a chip to permit a targeted molecular recognition by the corresponding cellular receptors to take place (4,5).

Therefore, several technical difficulties must be conquered to establish a high-throughput carbohydrate microarray technology. These difficulties take into account whether carbohydrate macromolecules of hydrophilic character can be immobilized on a chip surface by methods that are suitable for high-throughput production of microarrays; whether immobilized carbohydrate-containing macromolecules preserve their immunological properties, such as expression of carbohydrate-epitopes or antigenic determinants and their solvent accessibility; whether the carbohydrate microarray system reaches the sensitivity, specificity, and capacity to detect a broad range of antibody specificities in clinical specimens; and eventually whether this technology can be applied to investigate the carbohydrate-mediated molecular recognition on a titanic scale that was previously impossible.

3. Experimental Approach to Establishment of High-Throughput Carbohydrate Microarrays

Our intent was to introduce immunological specificity to microarray technology to establish a microarray-based broad-range immunosensor for the exploration of immunological diversity of carbohydrates and the immune responses to carbohydrate antigens. In experimental design, we applied a well-studied model system of carbohydrate–anticarbohydrate interaction, $\alpha(1,6)$ dextran and anti- $\alpha(1,6)$ dextran antibodies (25–27), for our initial investigation (1). To address whether carbohydrate macromolecules of hydrophilic character can be immobilized on a chip surface, we applied the fluorescein isothiocyanate (FITC)-conjugated $\alpha(1,6)$ dextran as probes to screen available chip substrates that were produced for printing cDNA microarrays for their potential use in carbohydrate microarrays. This investigation guided us to the discovery that the nitrocellulose-coated glass slides are suitable for immobilization of carbohydrate-containing macromolecules.

To test whether the size and molecular weight of polysaccharides influence their surface immobilization, FITC- $\alpha(1,6)$ dextran preparations of different molecular weights and of similar molar ratios of FITC/glucose were applied. A structurally distinct polysaccharide, inulin, was chosen as a control to see whether surface immobilization of polysaccharides is restricted to a specific carbohydrate structure. This investigation demonstrated that dextran preparations of different molecular weights, ranging from 20 to 2000 kDa, and inulin of 3.3 kDa could be printed and immobilized on the nitrocellulose-coated slide without chemical conjugation. The linear range of the material transferred and surface immobilized, however, differs significantly among dextran preparations of different molecular weights (1).

To investigate whether immobilized carbohydrate antigens preserve their antigenic determinants, dextran preparations of different linkage compositions and with different ratios of terminal to internal epitopes were printed on nitrocellulose-coated glass slides. These preparations included N279, displaying both internal linear and terminal nonreducing end epitopes, B1299S, heavily branched and expressing predominantly terminal epitopes, and LD7, a synthetic dextran composed of 100% $\alpha(1,6)$ -linked internal linear chain structure. The dextran microarrays were incubated with monoclonal antibodies of defined specificities, either a groove-type anti- $\alpha(1,6)$ dextran 4.3F1 (IgG3) (28) or a cavity-type anti- $\alpha(1,6)$ dextran 16.4.12E (IgA) (29). The former recognizes the internal linear chain of $\alpha(1,6)$ dextran, whereas the latter is specific for the terminal nonreducing end structure of the polysaccharide.

The groove-type monoclonal antibody (mAb), 4.3F1, bound well to the dextran preparations with predominantly linear chain structures, N279 and LD7, but bound poorly to the heavily branched $\alpha(1,6)$ dextran, B1299S. By contrast, when the cavity-type mAb 16.4.12E was applied, it bound to the immobilized dextran preparations with branches (N279 and B1299S) but not to those with only internal linear chain structure (LD7). These patterns of antigen–antibody reactivities are typically identical to those recognized by an ELISA binding assay for either the groove type or cavity type of anti-dextran mAbs. Therefore, the immunological properties of dextran molecules are well preserved when immobilized on a nitrocellulose-coated glass slide. Their nonreducing end structure, recognized by the cavity-type anti- $\alpha(1,6)$ dextran as well as the internal linear chain epitopes bound by the groove-type anti- $\alpha(1,6)$ dextran are displayed on

the surface after immobilization and are accessible to antibodies in an aqueous solution. This approach was then extended to test a large panel of carbohydrate-containing macromolecules to assess their immobilization on chip and to evaluate expression of antigenic structures for antibody detection. We demonstrated that polysaccharides and glycoconjugates of distinct structural configurations and of diverse sugar chain contents were applicable for this biochip platform, i.e., a method of nitrocellulose-based noncovalent immobilization for high-throughput construction of carbohydrate microarrays (1).

Nitrocellulose polymer is a fully nitrated derivative of cellulose in which free hydroxyl groups are substituted by nitro groups, and it is thus hydrophobic in character. Documented investigations have suggested that immobilization of proteins on a nitrocellulose membrane requires revelation of their hydrophobic surfaces to the membrane (30,31). The molecular forces for the carbohydrate–nitrocellulose interaction remain to be characterized. Perhaps the 3D microporous configuration of the nitrocellulose coating on the slides, the macropolymer characteristics of polysaccharides and the polyamphiphathic properties of many carbohydrate-containing macromolecules are key factors for the stable immobilization of carbohydrate antigens on the slide. Given the structural diversity of carbohydrate antigens, we highly recommend that each preparation must be tested on this chip substrate.

4. Practical Platform of Carbohydrate Microarrays

The aforementioned experimental investigations have guided our research to the establishment of a high-throughput platform of carbohydrate microarrays. As illustrated in the **Fig. 1**, this approach applies to existing cDNA microarray systems, including spotter and scanner, for carbohydrate array production and applications. A key technical element of this array platform is the introduction of nitrocellulose-coated microglass slides to immobilize unmodified carbohydrate antigens on the chip surface.

A high-precision robot designed to produce cDNA microarrays was used to spot carbohydrate antigens onto a chemically modified glass slide. The microspotting capacity of this system is approx 20,000 spots per chip. The antibody-stained slides were then scanned for fluorescent signals with a Biochip scanner that was developed for cDNA microarrays.

For microspotting, antigens and antibodies were printed using PIXSYS 5500C (Cartesian Technologies, Irvine, CA). Supporting substrate was FAST Slides (Whatman Schleicher and Schuell, Keene, NH). For immunofluorescence staining, the staining procedure used is essentially identical to regular immunofluorescent staining of tissue sections. For microarray scanning, a ScanArray 5000 Standard Biochip Scanning System and its QuantArray software (Perkin-Elmer, Boston, MA) were used for scanning and data capture.

4.1. An Eight-Chamber Subarray System for Customized Arrays

We have designed an eight-chamber subarray system to create customized carbohydrate microarrays for defined purposes. As illustrated in the **Fig. 2**, each microglass slide contains eight separated subarrays. The microarray capacity is approx 600 microspots per subarray. A single slide is designed to enable eight microarray analyses. A similar design with array capacity of approx 100 microspots is also commer-

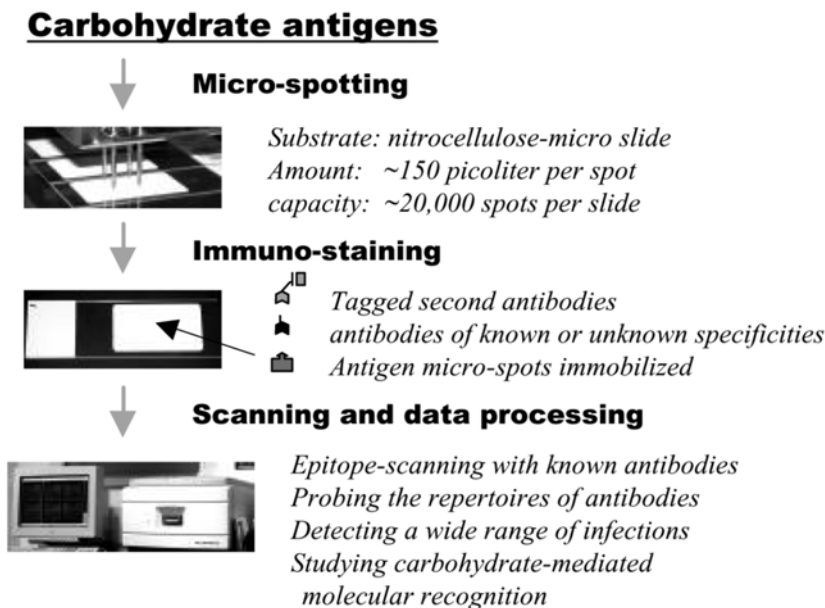


Fig. 1. A high-throughput biochip platform for constructing carbohydrate-based microarrays.

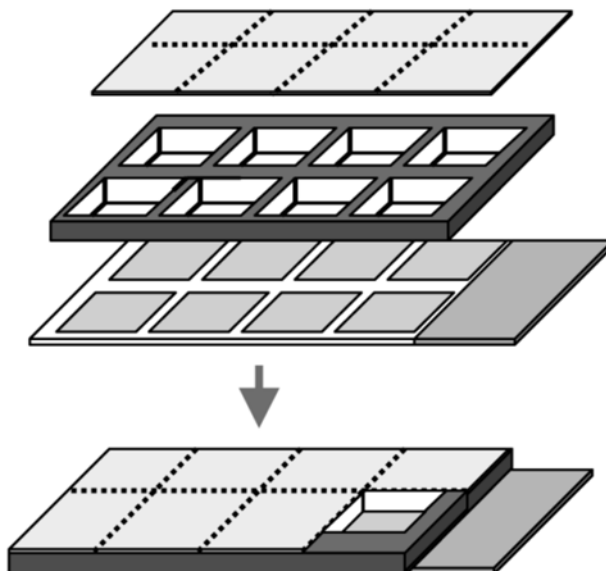


Fig. 2. Graphical presentation of the eight-chamber subarrays.

cially available (Whatman Schleicher and Schuell). For eight-chamber subarrays. Each microglass slide contains eight subarrays of identical content. There is chip space for 600 microspots per subarray, with spot sizes of approx 200- and 300- μm intervals, center to center. A single slide is, therefore, designed to permit eight detections. For

repeats and dilutions, each antigen will be printed at 0.5–1.0 mg/mL and also at a 1:10 dilution of the initial concentration. A given concentration of each preparation will be repeated at least three times to allow statistical analysis of detection of identical preparation at given antigen concentration. For antibody isotype standard curves, antibodies of IgG, IgA, and IgM isotype of corresponding species serve as standard curves for antibody detection and normalization.

4.2. Examination of the Presence of Antigens and Antibodies on the Array

To verify that we have successfully “printed” proteins, synthetic peptides, and carbohydrates, we incubate microarrays with antibodies, receptors, or lectins known to react with the printed substance. The reaction is detected either directly by conjugating with a fluorochrome to the “detector” or by a second-step staining procedure.

4.3. Staining and Scanning of Carbohydrate Microarrays

Immediately before use, the printed microarrays are rinsed with phosphate-buffered saline (PBS), pH 7.4, with 0.05% Tween 20 and then blocked by incubating the slides in 1% bovine serum albumin in PBS containing 0.05% NaN₃ at 37°C for 30 min. They are then incubated at room temperature with serum specimens at given dilutions in 1% bovine serum albumin PBS containing 0.05% NaN₃ and 0.05% Tween-20. Next, anti-human (or other species) IgG, IgM, or IgA antibodies with distinct specific fluorescent tags (Cy3, Cy5, or FITC) are applied to reveal the bound antibodies according to their Ig heavy chain isotypes. The stained slides are rinsed five times with PBS with 0.05% Tween-20 after each staining step. ScanArray 5000A is used to scan the microarray. This instrument is a standard biochip scanning system (Perkin-Elmer) equipped with multiple lasers, emission filters and ScanArray acquisition software.

4.4. Analysis of Microarray Data

Fluorescence intensity values for each array spot and its background are calculated using QuantArray software analysis packages. Data for at least three replicates for each substance analyzed are collected on each chip (“triple spotting”).

4.5. Validation and Further Investigation of Microarray Observations

It is always astute to verify microarray data by other experimental approaches. We check our carbohydrate microarray findings by doing conventional immunoassays, e.g., ELISA, dot blot, Western blot, flow cytometry, and immunohistology. Examples of such investigations were described in our recent publications (1,3).

5. Promising Areas for Exploring Carbohydrate Microarray Technology

As described above, carbohydrates of multiple molecular configurations and of diverse sugar chain structures can be stably immobilized on a nitrocellulose-coated glass slide without chemical conjugation (1,3). A direct application of this technology is in exploring the repertoires of human antibodies with anticarbohydrate activities. When a large collection of microbial carbohydrate antigens is arrayed on a sugar chip, such array would allow a simultaneous detection and characterization of a wide range of antibody reactivities and provide specific diagnostic information of infectious diseases. In combination with the use of semisynthetic glycoconjugates, which display

unique oligosaccharide chains, a biochip-based characterization of the epitope-binding specificity of a single antibody or lectin and determination of the dominant antigenic responses elicited by a natural infection or a vaccination would be practically achievable. Similarly, this technology can be extended to monitor autoantibodies and tumor-specific or -associated anticarbohydrate activities.

This novel approach can be applied to other biological systems in which a carbohydrate-carbohydrate or carbohydrate-protein interaction plays a significant role. For example, a sugar chip can be applied in screening for the carbohydrate-based cellular receptors of a microorganism. Experimentally, a candidate protein or the whole cell of a microbe can be placed on a sugar chip to probe the carbohydrate structures that a microbial pathogen may bind and selectively colonize in certain type of host cells or tissue environments. Such investigation is noteworthy, because it may lead to a better understanding of the host-microbe biological relationship as well as the pathogenesis mechanism of human pathogens. A reverse type of application is to print a large panel of structurally uncharacterized polysaccharides or glycoconjugates, such as those isolated from mixtures of natural herbs of traditional eastern medicines, to react with antibodies or lectins of known carbohydrate-binding specificities. In this way, the sugar chain epitope profile of these printed preparations can be rapidly recognized, providing important clues for drug discovery. In addition, such sugar chain epitope mapping strategy is technically straightforward and is suitable to serve as a scanning method to verify and control the quality of a complex formula of herbal medicine or nutrition additives, which contain significant quantities of glycan components or lectins with carbohydrate-binding reactivities.

Recently, we have focused on the establishment of a glycomics strategy to facilitate identification of the sugar moieties of biological significance. We proposed to take advantage of the highly evolved immune systems of mammals to recognize the immunogenic sugar moieties of microbes. Specifically, we use carbohydrate microarrays to capture specific antibodies elicited by a microorganism (see **Fig. 3** for a schematic illustration of this approach). To critically evaluate this strategy, we have chosen a previous unrecognized viral pathogen, severe acute respiratory syndrome-associated coronavirus (SARS-CoV) (**32-34**), as a model for our investigation. This task was difficult and challenging, because information regarding the sugar moieties of this virus was entirely unavailable. This information is, however, very important for our consideration of vaccination strategy against SARS-CoV as well as investigation of pathogenic mechanisms of SARS. Therefore, we constructed glycan arrays to display carbohydrate antigens of defined structures and subsequently applied these tools to detect carbohydrate-specific antibody "fingerprints" that were elicited by a SARS vaccine. Our rationale was that if SARS-CoV expressed antigenic carbohydrate structures, then immunizing animals by using the whole virus-based vaccines would have the possibility to elicit antibodies specific for these structures. In addition, if SARS-CoV displayed a carbohydrate structure that mimics host cellular glycans, then vaccinated animals may develop antibodies with autoimmune reactivity to their corresponding cellular glycans.

By characterizing the SARS-CoV neutralizing antibodies elicited by an inactivated SARS-CoV vaccine, we detected autoantibody reactivity specific for the carbohydrate moieties of an abundant human serum glycoprotein asialo-orosomucoid (ASOR) (**Fig. 3B**).

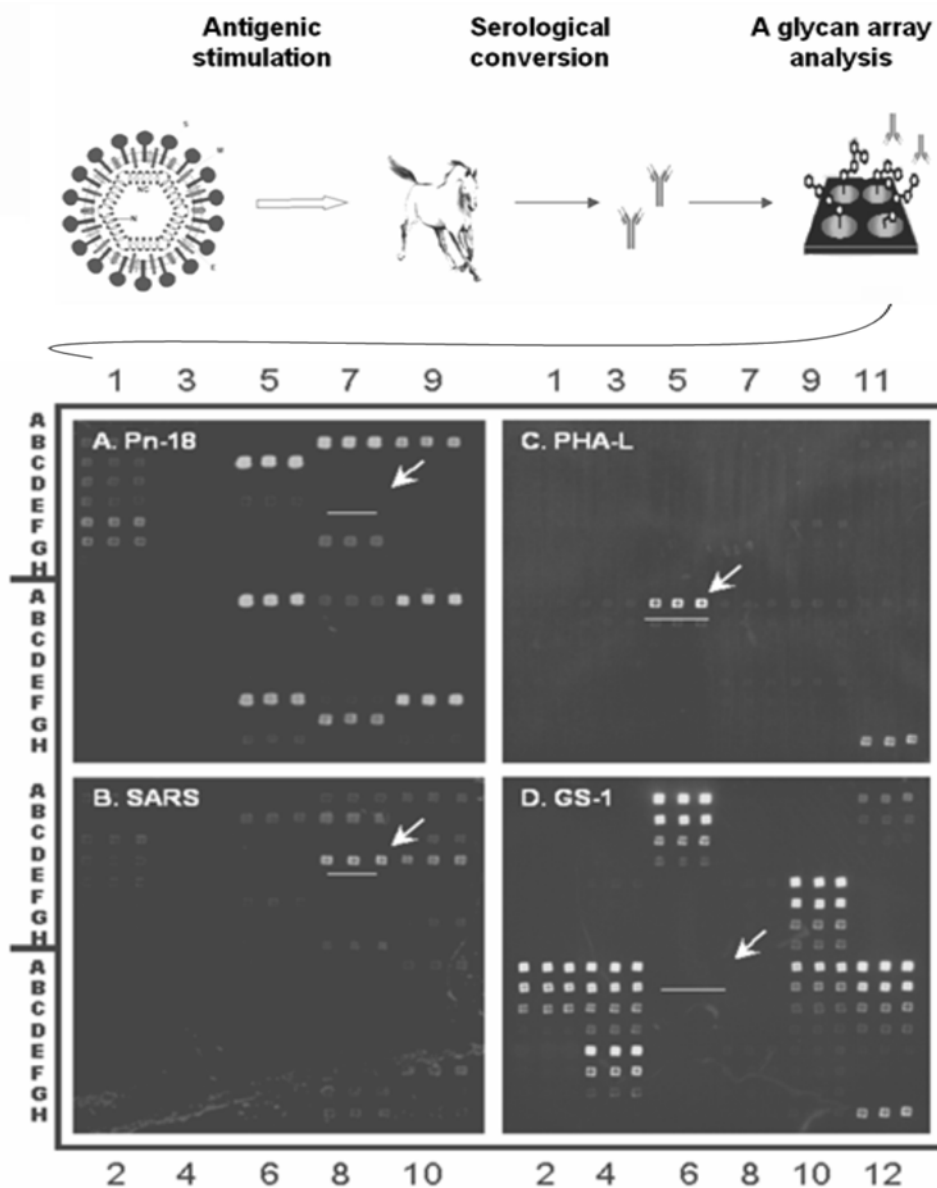


Fig. 3. Glycan arrays are used to characterize the antibody profiles of vaccinated animals (glycan array I) and to scan for ASOR-specific immunological probe (glycan array II). Antigen preparations spotted on each glycan array and their array locations are summarized in supplemental Tables S1 and S2 of **ref. 3** (available at the Physiological Genomics website at <http://physiolgenomics.physiology.org/cgi/content/full/00102.2004/DC1>).

For array I (**Fig. 3A,B**), a glycan array that contains 51 antigens (0.5 ng/microspot) was constructed and applied to scan horse anti-Pn 18 serum (**Fig. 3A**) as well as anti-SARS neutralizing antibodies (**Fig. 3B**).

For array II (**Fig. 3C,D**), a glycan array that displays 24 antigens, many of preparations of Gal-containing carbohydrate antigens, was stained by lectin PHA-L (**Fig. 3C**), which is specific for Gal β 1,4-*N*-acetylglucosamine-linked units, and by lectin GS1-B4 (**Fig. 3D**), which is considered to be specific for Gal α 1-3Gal.

This surveillance provides important clues for the selection of specific immunological probes to further examine whether SARS-CoV expresses antigenic structures that imitate the host glycan. We found that lectin PHA-L (for *Phaseolus vulgaris* L.) is specific for a defined complex carbohydrate of ASOR (**Fig. 3C,D**). Using this reagent as a probe, we confirmed that only the SARS-CoV-infected cells express a PHA-L-reactive antigenic structure (*see ref. 3* for the details). We obtained, therefore, immunologic evidences that a carbohydrate structure of SARS-CoV shares antigenic similarity with host glycan complex carbohydrates. This viral component is probably responsible for the stimulation of the autoantibodies directed at a cellular glycan complex carbohydrate.

These observations raise important questions about whether autoimmune responses are indeed elicited by SARS-CoV infection and whether such autoimmunogenicity contributes to SARS pathogenesis. ASOR is an abundant human serum glycoprotein and the ASOR-type complex carbohydrates are also expressed by other host glycoproteins (**35,36**). Thus, the human immune system is generally nonresponsive to these self-carbohydrate structures. However, when similar sugar moieties were expressed by a viral glycoprotein, their cluster configuration could differ significantly from those displayed by a cellular glycan, and in this manner, generate a novel nonself antigenic structure. A documented example of such antigenic structure is a broad-range HIV-1 neutralization epitope recognized by a monoclonal antibody 2G12. This antibody is specific for a unique cluster of sugar chains displayed by the gp120 glycoprotein of HIV-1 (**37**). It is, hence, important to examine whether naturally occurring SARS-CoV expresses the ASOR-type autoimmune reactive sugar moieties. During a SARS epidemic spread, the viruses replicate in human cells. Their sugar chain expression may differ from the monkey cell-produced viral particles. Scanning of the serum antibodies of SARS patients by using glycan arrays or other specific immunological tools may endow with information to shed light on this question.

In synopsis, recent establishment of carbohydrate-based microarrays, and especially the availability of different technological platforms to meet the multiple needs of carbohydrate research, marks an important developmental stage of postgenomic research (**1,20–24,38**). Our laboratory has established a simple, precise, and highly efficient experimental approach for the construction of carbohydrate microarrays (**1–3**). This approach makes use of existing cDNA microarray system, including spotter and scanner, for carbohydrate array production. A key technical element of this array platform is the introduction of nitrocellulose-coated microglass slides to immobilize unmodified carbohydrate antigens on the chip surface noncovalently. This technology has achieved the sensitivity to recognize the profiles of human anti-carbohydrate antibodies with as little as a few microliters of serum specimen and reached the chip capacity to include the antigenic preparations of most common pathogens (~20,000 microspots per biochip). We described also an eight-chamber subarray system to produce carbohydrate microarrays of relative smaller scale, which is more frequently applied in our laboratory's routine research activities. Of late, we applied this system to assemble glycan arrays to probe the immunogenic sugar moieties of a recently discovered viral pathogen, SARS-CoV. This research approach is probably applicable for the identification of immunological targets of other microorganisms and for the exploration of complex carbohydrates that are differentially expressed by host cells, including stem cells at various stages of differentiation and human cancers.

References

1. Wang, D., Liu, S., Trummer, B. J., Deng, C., and Wang, A. (2002) Carbohydrate microarrays for the recognition of cross-reactive molecular markers of microbes and host cells. *Nat. Biotechnol.* **20**, 275–281.
2. Wang, D. (2003) Carbohydrate microarrays. *Proteomics* **3**, 2167–2175.
3. Wang, D., and J. Lu. (2004) Glycan arrays lead to the discovery of autoimmunogenic activity of SARS-CoV. *Physiol. Genomics* 2004, **18**, 245–248.
4. Wang, D., and Kabat, E. A. (1996) Carbohydrate antigens (polysaccharides). In: *Structure of Antigens, Vol. 3* (M. H. V. Van Regenmortel, ed.), CRC Press, Boca Raton, FL, pp. 247–276.
5. Brooks, S. A., Dwek, M. V., and Schumacher, U. (2002) *Functional and Molecular Glycobiology*. BIOS Scientific Publishers Ltd., Oxford, United Kingdom.
6. Wang, D. (2004) Carbohydrate antigens. In: *Encyclopedia of Molecular Cell Biology and Molecular Medicine Vol. 2*, (R. A. Meyers, ed.), Wiley-VCH, pp. 277–301.
7. Feizi, T. (1982) The antigens Ii, SSEA-1 and ABH are in interrelated system of carbohydrate differentiation antigens expressed on glycosphingolipids and glycoproteins. *Adv. Exp. Med. Biol.* **152**, 167–177.
8. Hakomori, S. (1985) Aberrant glycosylation in cancer cell membranes as focused on glycolipids: overview and perspectives. *Cancer Res.* **45**, 2405–2414.
9. Focarelli, R., La Sala, G. B., Balasini, M., and Rosati, F. (2001) Carbohydrate-mediated sperm-egg interaction and species specificity: a clue from the *Unio elongatulus* model. *Cells Tissues Organs* **168**, 76–81.
10. Crocker, P. R., and Feizi, T. (1996) Carbohydrate recognition systems: functional triads in cell-cell interactions. *Curr. Opin. Struct. Biol.* **6**, 679–691.
11. Heidelberger, M., and Avery, O. T. (1923) The soluble specific substance of *Pneumococcus*. *J. Exp. Med.* **38**, 73–80.
12. Dochez, A. R., and Avery, O. T. (1917) The elaboration of specific soluble substance by pneumococcus during growth. *J. Exp. Med.* **26**, 477–493.
13. Ezzell, J. W., Jr., Abshire, T. G., Little, S. F., Lidgerding, B. C., and Brown, C. (1990) Identification of *Bacillus anthracis* by using monoclonal antibody to cell wall galactose-N-acetylglucosamine polysaccharide. *J. Clin. Microbiol.* **28**, 223–231.
14. Robbins, J. B., and Schneerson, R. (1990) Polysaccharide-protein conjugates: a new generation of vaccines. *J. Infect. Dis.* **161**, 821–832.
15. Mond, J. J., Lees, A., and Snapper, C. M. (1995) T cell-independent antigens type 2. *Annu. Rev. Immunol.* **13**, 655–692.
16. DeRisi, J., Penland, L., Brown P. O., et al. (1996) Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat. Genet.* **14**, 457–460.
17. Brown, P. O., and Botstein, D (1999) Exploring the new world of the genome with DNA microarrays. *Nat. Genet.* **21(Suppl. 1)**, 33–37.
18. MacBeath, G., and Schreiber, S. L. (2000) Printing proteins as microarrays for high-throughput function determination (see comments). *Science* **289**, 1760–1763.
19. Stoll, D., Templin, M. F., Schrenk, M., Traub, P. C., Vohringer, C. F., and Joos, T. O. (2002) Protein microarray technology. *Front. Biosci.* **7**, C13–C32.
20. Willats, W. G., Rasmussen, S. E., Kristensen, T., Mikkelsen, J. D., and Knox, J. P. (2002) Sugar-coated microarrays: a novel slide surface for the high-throughput analysis of glycans. *Proteomics* **2**, 1666–1671.
21. Fazio, F., Bryan, M. C., Blixt, O., Paulson, J. C., and Wong, C. H. (2002) Synthesis of sugar arrays in microtiter plate. *J. Am. Chem. Soc.* **124**, 14,397–14,402.
22. Fukui, S., Feizi, T., Galustian, C., Lawson, A. M., and Chai, W. (2002) Oligosaccharide microarrays for high-throughput detection and specificity assignments of carbohydrate-protein interactions. *Nat. Biotechnol.* **20**, 1011–1017.

23. Houseman, B. T., and Mrksich, M. (2002) Carbohydrate arrays for the evaluation of protein binding and enzymatic modification. *Chem. Biol.* **9**, 443–454.
24. Park, S., and Shin, I. (2002) Fabrication of carbohydrate chips for studying protein-carbohydrate interactions. *Ang. Chem. Int. Ed. Engl.* **41**, 3180–3182.
25. Kabat, E. A., and Berg, D. (1953) Dextran–an antigen in man. *J. Immunol.* **70**, 514–532.
26. Cisar, J., Kabat, E. A., Dorner, M. M., Liao, J. (1975) Binding properties of immunoglobulin combining sites specific for terminal or nonterminal antigenic determinants in dextran. *J. Exp. Med.* **142**, 435–459.
27. Wang, D., Liao, J., Mitra, D., Akolkar, P. N., Gruezo, F., and Kabat, E. A. (1991) The repertoire of antibodies to a single antigenic determinant. *Mol. Immunol.* **28**, 1387–1397.
28. Wang, D., Chen, H. T., Liao, J., et al. (1990) Two families of monoclonal antibodies to $\alpha(1,6)$ dextran, V_H19.1.2 and V_H9.14.7, show distinct patterns of J_k and J_H minigene usage and amino acid substitutions in CDR3. *J. Immunol.* **145**, 3002–3010.
29. Matsuda, T., and Kabat, E. A. (1989) Variable region cDNA sequences and antigen binding specificity of mouse monoclonal antibodies to isomaltosyl oligosaccharides coupled to proteins T-dependent analogues of $\alpha(1,6)$ dextran. *J. Immunol.* **142**, 863–870.
30. Oehler, S., Alex, R., and Barker, A. (1999) Is nitrocellulose filter binding really a universal assay for protein-DNA interactions? *Anal. Biochem.* **268**, 330–336.
31. Van Oss, C. J., Good, R. J., and Chaudhury, M. K. (1987) Mechanism of DNA (Southern) and protein (Western) blotting on cellulose nitrate and other membranes. *J. Chromatogr.* **391**, 53–65.
32. Fouchier, R. A., Kuiken, T., Schutten, M., et al. (2003) Aetiology: Koch's postulates fulfilled for SARS virus. *Nature* **423**, 240.
33. Ksiazek, T. G., Erdman, D., Goldsmith, C. S., et al. (2003) A novel coronavirus associated with severe acute respiratory syndrome. *N. Engl. J. Med.* **348**, 1953–1966.
34. Rota, P. A., Oberste, M. S., Monroe, S. S., et al. (2003) Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* **300**, 1377–1380.
35. Cummings, R. D., and Kornfeld, S. (1984) The distribution of repeating (Gal beta 1,4GlcNAc beta 1,3) sequences in asparagine-linked oligosaccharides of the mouse lymphoma cell lines BW5147 and PHAR 2.1. *J. Biol. Chem.* **259**, 6253–6260.
36. Pacifico, F., Montuori, N., Mellone, S., et al. (2003) The RHL-1 subunit of the asialoglycoprotein receptor of thyroid cells: cellular localization and its role in thyroglobulin endocytosis. *Mol. Cell. Endocrinol.* **208**, 51–59.
37. Calarese, D. A., Scanlan, C. N., Zwick, M. B., et al. (2003) Antibody domain exchange is an immunological solution to carbohydrate cluster recognition. *Science* **300**, 2065–2071.
38. Adams, E. W., Ratner, D. M., Bokesch, H. R., McMahon, J. B., O'Keefe, B. R., and Seeberger, P. H. (2004) Oligosaccharide and glycoprotein microarrays as tools in HIV glycobiology; glycan-dependent gp120/protein interactions. *Chem. Biol.* **11**, 875–881.