Glycobiology, 2016, vol. 26, no. 5, 443–448
doi: 10.1093/glycob/cwv171
Advance Access Publication Date: 10 January 2016
Communication

OXFORD

Glycan Recognition

Diverse molecular recognition properties of blood group A binding monoclonal antibodies

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Received 5 October 2015; Revised 17 December 2015; Accepted 24 December 2015

Abstract

Information about specificity and affinity is critical for use of carbohydrate-binding antibodies. Herein, we evaluated eight monoclonal antibodies to the blood group A (BG-A) antigen. Antibodies 87-G, 9A, HE-10, HE-24, HE-193, HE-195, T36 and Z2A were profiled on a glycan microarray to assess specificity, relative affinity and the influence of glycan density on recognition. Our studies highlight several noteworthy recognition properties. First, most antibodies bound GalNAcα1–3Gal and the BG-A trisaccharide nearly as well as larger BG-A oligosaccharides. Second, several antibodies only bound the BG-A trisaccharide when displayed on certain glycan chains. These first two points indicate that the carrier glycan chains primarily influence selectivity, rather than binding strength. Third, binding of some antibodies was highly dependent on glycan density, illustrating the importance of glycan presentation for recognition. Fourth, some antibodies recognized the tumor-associated Tn antigen, and one antibody only bound the variant composed of a GalNAc-alpha-linked to a serine residue. Collectively, these results provide new insights into the recognition properties of anti-BG-A antibodies.

Key words: Anti-glycan antibodies, blood group antigen, glycan microarray

Introduction

Carbohydrate-binding antibodies play a key role in basic research and a number of them have applications as diagnostics and therapeutics. A prime example are antibodies to the blood group A (BG-A) antigen, a glycan determinant found on various human cells and secreted glycoproteins (Storry and Olsson 2009). BG-A expression is a key consideration when evaluating compatibility for blood transfusions and organ transplants, and altered expression occurs during various disease states such as cancer (Storry and Olsson 2009). Monoclonal antibodies to the BG-A antigen are used at length for ABO blood typing (Malomgré and Neumeister 2009) and for monitoring expression in various tissues (for some recent examples, see Jeyakanthan et al. (2015), Lindberg et al. (2013) and Miura et al. (2013)). Beyond monoclonal antibodies, serum antibodies to BG-A are evaluated when conducting reverse blood typing (Malomgré and Neumeister 2009) and are potential biomarkers for a cancer vaccine (Campbell et al. 2013).

Information about specificity is crucial for proper selection of antibodies and interpretation of results. At a molecular level, recognition of BG-A can be complex. The minimal structure that defines the BG-A determinant is the trisaccharide GalNAcα1–3(Fucα1–2)Gal. In nature, this trisaccharide is appended to various carrier glycan chains resulting in six different tetrasaccharides (BG-A1 through A6, see Table I and Supplementary data, Figure S1). These tetrasaccharides are attached to glycan chains of varying length and composition on both glycolipids and glycoproteins. Thus, the BG-A trisaccharide can be displayed in numerous contexts. Monoclonal antibodies can vary in their ability to recognize BG-A in different settings, such as preferentially binding BG-A displayed on certain carrier chains (Clausen and Hakomori 1989; Lindberg et al. 2011, 2012; Jeyakanthan et al. 2015). Finally, a number of natural glycan determinants are structurally similar to BG-A, and information about potential reactivity with these glycans is critical. Although many prior studies have evaluated specificity of antibodies to BG-A [for some examples, see Chen and Kabat (1985), Furukawa et al. (1985), Gooi et al. (1985), Némec et al. (1987), Oriol et al. (1990), Barr et al. (2014, 2015) and references cited therein], these studies typically focused on a limited number of BG-A variants and relatively few non-BG-A structures.

Table I. Core/backbone types

Carrier/backbone name	Glycan sequence
Type 1	~β1–3GlcNAcβ-
Type 2	~β1–4GlcNAcβ-
Type 3	~β1–3GalNAcα1-
Type 4	~β1–3GalNAcβ1-
Type 5	~β1–3Galβ1-
Type 6	~β1–4Glcβ1-
2′F-type 2	~β1–4(Fucα1–3)GlcNAcβ-
LeB	~β1–3(Fucα1–4)GlcNAcβ1–3Galβ1-
Globo	~β1–3GalNAcβ1–3Galα1–4Galβ1-

Glycan microarrays allow high-throughput evaluation of antibody binding to numerous different glycan sequences (Park et al. 2013; Arthur et al. 2014; Palma et al. 2014). Herein, we used a microarray with 384 components to evaluate binding properties of eight antibodies to BG-A. In addition to a diverse collection of N-linked, O-linked and glycolipid glycans, the microarray contained many variants of BG-A, blood group B (BG-B), blood group H (BG-H), the Tn antigen (GalNAcα-Ser/Thr), core 5 and the Forssman antigen. Furthermore, the array also included variations in glycan density. The studies provide new insights into the binding properties of BG-A antibodies.

Results

Initial screening

After surveying the literature and antibody databases, we found 71 monoclonal antibodies to the BG-A antigen, of which 29 were commercially available. We selected eight commercially available antibodies (six IgM and two IgG) for an initial screen on our glycan microarray: 87-G, 9A, HE-10, HE-24, HE-193, HE-195, T36 and Z2A. Each was evaluated at a dilution of 1:50 from the original stock (Figure 1), a useful dilution for many applications. All of the antibodies bound at least one BG-A variant, but none had the exact same binding profile.

87-G (IgM)

87-G bound well to BG-A on type 2 and type 6 chains, regardless of glycan density. While 87-G bound poorly to BG-A on type 1, 3, 4 and 5 chains, it bound well to the BG-A trisaccharide and the disaccharide substructure of BG-A, GalNAcα1–3Gal. Antibody 87-G was found to bind strongly to the terminal disaccharide of the Forssman antigen (GalNAcα1–3GalNAcβ), but no binding was observed to the tetrasaccharide (GalNAcα1–3GalNAcβ1–3Galα1–4Galβ-) or glycopeptides containing core 5 (GalNAcα1–3GalNAcα1-Ser). The production and characterization of 87-G has not been published; however, it is reported to bind BG-A1 and BG-A2 by the commercial supplier (see Supplementary data, Table SI).

T36 (IgG) and HE-195 (IgM)

Antibodies T36 (Furukawa et al. 1985) and HE-195 had very similar selectivity at a dilution of 1:50. Both antibodies bound well to all variants of the BG-A antigen and GalNAcα1–3Gal. Density did not affect binding of these antibodies at a dilution of 1:50, but density-dependent binding was observed for T36 at lower concentrations (see below). Neither antibody bound any non-BG-A antigens. T36 is known to bind BG-A1, A2 and A3 (Furukawa et al. 1985), but recognition of BG-A4, A5 and A6 has not been previously reported. The production and characterization of HE-195 have not been published.

HE-10 (IgM)

HE-10 (Vanak et al. 1989) showed the narrowest specificity. It bound well to BG-A on a type 3 or type 4 chain but had negligible binding to other BG-A variants. It showed modest binding (about 10-fold lower signals) to BG-B on a type 3 or 4 chain. HE-10 displayed a modest (3- to 4-fold difference in signal) preference for high density glycans, but binding to low density neoglycoproteins was readily apparent. HE-10 did not bind any non-BG-A or BG-B antigens. Previously, HE-10 was found to bind BG-A3, BG-A4, BG-A5, BG-B3, BG-B4, BG-B5, BG-H3, BG-H4 and BG-H5 (Némec et al. 1987). While we observed selectivity for BG-A3 and A4, we did not observe binding to BG-A5, B5, H3, H4 or H5. Each of these glycans is accessible for binding on our array (Muthana et al. 2015). The difference between our results and those of Némec *et al.* may be due to their use of a higher antibody concentration.

Z2A (IgM)

Z2A bound well to all BG-A variants except BG-A on a Lewis B chain (A-LeB), which is notable given that BG-A1 is a substructure of A-LeB (see Table II and Supplementary data, Figure S1). It also bound well to GalNAcα1–3Gal. Little or no density preference was observed. Z2A was found to have some reactivity to the Forssman antigen, especially the terminal disaccharide. The production and characterization of Z2A have not been published; however, it is reported to bind BG-A1 and BG-A2 by a commercial supplier.

HE-193 (IgM)

HE-193 bound well to all BG-A variants regardless of carrier chain or glycan density. Strong binding was also observed to GalNAcα1–3Gal. HE-193 reacted substantially with the Forssman antigen (disaccharide and tetrasaccharide) as well as glycopeptides containing the core 5 glycan. The binding profile for BG-A variants and reactivity with the Forssman disaccharide is consistent with a previous report (Némec et al. 1987). Recognition of the terminal tetrasaccharide of the Forssman antigen and glycopeptides containing the core 5 glycan has not been previously reported.

HE-24 (IgM)

HE-24 (Némec et al. 1987) bound to all BG-A variants, but it did not bind to GalNAcα1–3Gal. It displayed a substantial preference for BG-A antigens presented at high density over low density. Antibody HE-24 reacted strongly with glycopeptides displaying the Tn antigen, but only when the GalNAc was attached to serine. HE-24 also bound asialo-ovine submaxillary mucin (aOSM), confirming recognition of Tn as presented naturally on a glycoprotein. While it did not bind Forssman-related glycans or core 5, it did bind GalNAcα1–6Gal. The binding profile for BG-A variants is consistent with a previous report (Némec et al. 1987), but the dependence on glycan density, recognition of the Tn antigen and binding to GalNAcα1–6Gal have not been previously studied.

9A (IgG)

9A (Parker et al. 1984) showed the broadest reactivity on the array. 9A bound well to all BG-A variants except Globo A, which is notable given that it contains the BG-A4 tetrasaccharide at the non-reducing end (see Table II and Supplementary data, Figure S1). It also bound well to GalNAcα1–3Gal. This antibody showed a strong dependence on glycan density, with as much as 100-fold higher signals to high density variants relative to low density variants. Antibody 9A also

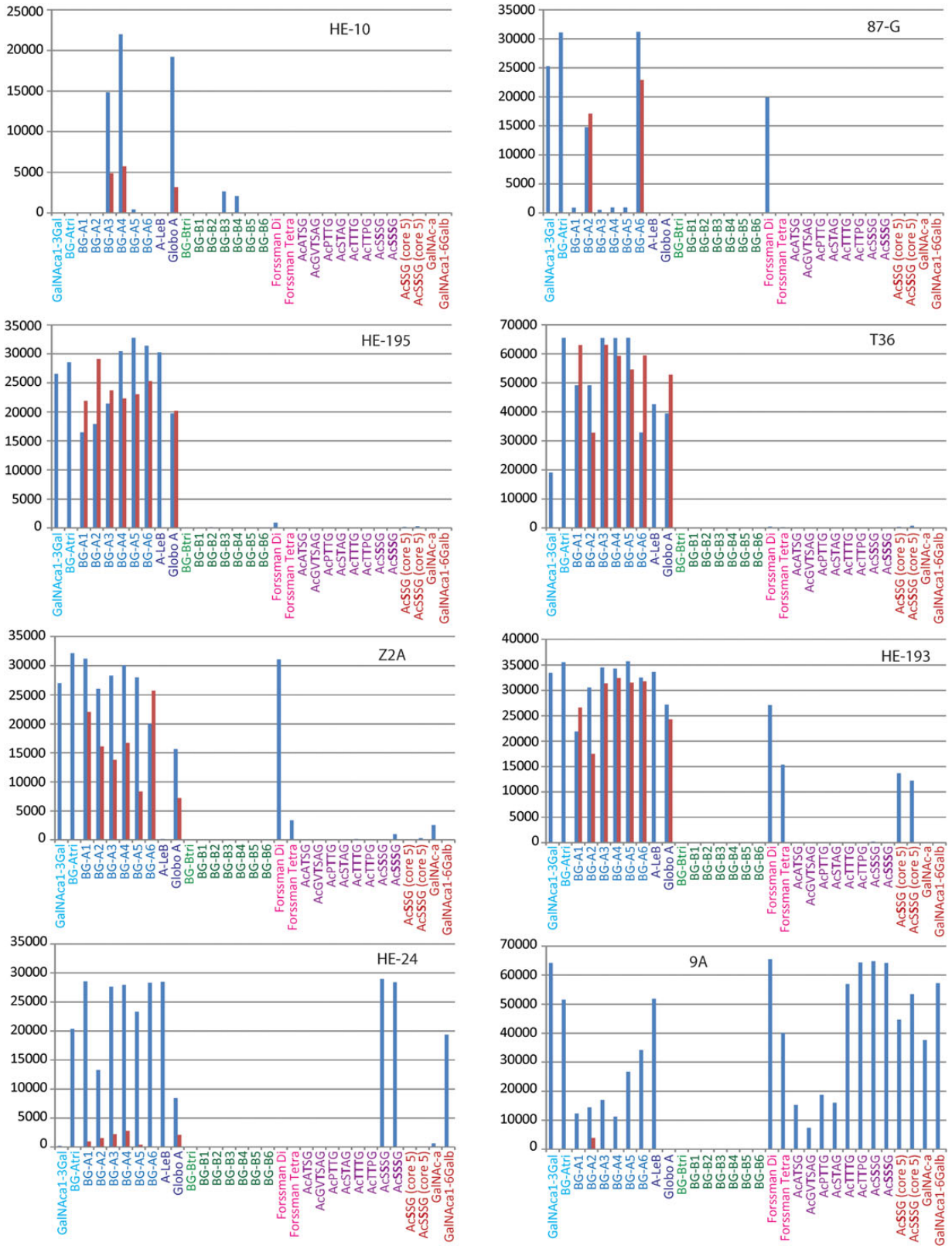

Fig. 1. Binding profiles of BG-A antibodies. Antibodies were tested at a dilution of 1:50. Bars represent raw fluorescence units (RFU). Blue bars depict signals from the high density variants. Data for low density variants are only shown for BG-A1 through A6 and Globo A (maroon bars). For glycopeptides, the glycosylated residue is highlighted in bold and contains a GalNAc-alpha linked unless otherwise listed (e.g. core 5). This figure is available in black and white in print and in color at *Glycobiology* online.

Table II. Apparent K_d values (μg/mL) for selected antibodies[a]

Abbreviation	Sequences	Density =	87-G Low/high[b]	T36 Low/high[b]	HE-195 Low/high[b]	Z2A Low/high[b]	HE-24 Low/high[b]
GalNAcα1–3Gal	GalNAcα1–3Galβ-		Weak/**0.32**	Weak/~**0.20**	Weak/**0.58**	**0.12/0.03**	NB/NB
BG-A	GalNAcα1–3(Fucα1–2)Galβ-		nt/**0.14**	nt/**0.013**	nt/**0.14**	nt/**0.0.03**	nt/**0.07**
BG-A1	GalNAcα1–3(Fucα1–2)Galβ1–3GlcNAcβ-		NB/weak	**0.013/0.002**	**0.05/0.04**	Weak/**0.44**	NB/**0.06**
BG-A2	GalNAcα1–3(Fucα1–2)Galβ1–4GlcNAcβ-		**0.55/0.19**	**0.025/0.003**	**0.05/0.04**	**0.03/0.02**	Weak/**0.02**
BG-A3	GalNAcα1–3(Fucα1–2)Galβ1–3GalNAcα1-		NB/weak	**0.05/0.004**	**0.06/0.04**	Weak/**1.7**	Weak/**0.01**
BG-A4	GalNAcα1–3(Fucα1–2)Galβ1–3GalNAcβ1-		NB/weak	**0.041/0.002**	**0.06/0.04**	Weak/**0.47**	Weak/**0.02**
BG-A5	GalNAcα1–3(Fucα1–2)Galβ1–3Galβ1-		NB/weak	**0.13/0.005**	**0.09/0.03**	Weak/**0.24**	Weak/**0.03**
BG-A6	GalNAcα1–3(Fucα1–2)Galβ1–4Glcb1-		**0.47/0.12**	**0.37/0.002**	**0.06/0.04**	**0.04/0.02**	NB/**0.06**
2′F-A type 2-Sp	GalNAcα1–3(Fucα1–2)Galβ1–4(Fucα1–3)GlcNAcβ-		**5.50/0.92**	**0.002/0.001**	**0.05/0.05**	**0.08/0.04**	**0.02/0.005**
A-LeB	GalNAcα1–3(Fucα1–2)Galβ1–3(Fucα1–4)GlcNAcβ1–3Galβ1-		NB/nt	**0.002**/nt	**0.05**/nt	NB/nt	**0.02**/nt
Globo A	GalNAcα1–3(Fucα1–2)Galβ1–3GalNAcβ1–3Galα1–4Galβ1-		NB/NB	**0.08/0.03**	**0.06/0.05**	Weak/**4.18**	Weak/**0.02**
Ac-S-S*-S-G	AcSer-(GalNAcα)Ser-Ser-Gly-Hex-		NB/NB	NB/NB	NB/NB	NB/NB	Weak/**0.008**
Forssman Di	GalNAcα1–3GalNAcβ1-		**1.4/0.20**	NB/NB	NB/NB	**0.07/0.02**	NB/NB
Forssman Tetra	GalNAcα1–3GalNAcβ1–3Galα1–4Galβ-		NB/NB	NB/NB	NB/NB	NB/weak	NB/NB
GalNAca1–6Galb	GalNAcα1–6Galβ-		NB/NB	NB/NB	NB/NB	NB/NB	NB/**0.20**

[a]Antibodies were assayed at a range of concentrations to produce a dose–response curve and the apparent K_d values (μg/mL) were determined for each glycan. Bold indicates substantial binding; NB, no binding; weak, some binding detectable at high concentrations, but signal is too low to determine an apparent K_d value; nt, not tested. Full array data can be found in the Supplementary Data, Excel file.

[b]Results for the glycan at low density are on the left and high density are on the right.

recognized the Forssman antigen (disaccharide and tetrasaccharide), many different Tn containing glycopeptides (both GalNAc-Ser and GalNAc-Thr), core 5 glycopeptides, GalNAc and the GalNAcα1–6Gal disaccharide. Antibody 9A bound aOSM, confirming recognition of Tn when presented naturally on a glycoprotein. Although little has been published on the specificity of 9A, this antibody has been profiled previously on a different glycan microarray produced by the Consortium for Functional Glycomics (CFG). On the CFG array, 9A bound well to 2′F-A tetra type 2 [GalNAcα1–3(Fucα1–2)Galβ1–4(Fucα1–3)GlcNAcβ] but had little or no binding to BG-A1, A2, A3, A6 or the disaccharide GalNAcα1–3Gal. Weak binding to the Forssman disaccharide was observed. BG-A4, BG-A5, Globo A and A-LeB were not present on that array. The differences in binding profiles are likely due to differences in glycan density on the two arrays. Binding signals on the CFG array are most similar to results with low density glycans in our array (Wang et al. 2014). If one only considers our low density variants, the binding profiles are consistent on the two arrays.

Follow-up screening

Next, we selected five antibodies for profiling at a range of concentrations to generate dose–response curves. The apparent K_d values were then determined for each glycan (see Table II).

87-G

The preference for BG-A on type 2 and 6 chains observed at 1:50 was even more pronounced in the dilution series. This antibody displayed over a 100-fold preference for type 2 and type 6 variants over type 1, 3, 4 and 5. Recognition of GalNAcα1–3Gal, the BG-A trisaccharide and the Forssman disaccharide were nearly as good as BG-A2 and BG-A6, but no signal was observed at any concentration for the Forssman tetrasaccharide.

T36

The binding profiles for T36 at low concentrations revealed some density-dependent binding properties that were not observed at 1:50. Preferences for high density variants ranged from 2- to 200-fold, depending on the particular BG-A variant. The dilution series also indicated that binding to GalNAcα1–3Gal (high density) was about 15-fold worse than binding to the BG-A trisaccharide.

HE-195

The binding properties of HE-195 in the dilution series were very similar to the profile at 1:50.

Z2A

While Z2A bound well to all BG-A variants at a dilution of 1:50, the dilution series revealed a 10- to 80-fold preference for BG-A2 and BG-A6 over type 1, 3, 4 and 5 variants. Binding to GalNAcα1–3Gal, the BG-A trisaccharide and the Forssman disaccharide was nearly as good as binding to the larger oligosaccharides, BG-A2 and BG-A6.

HE-24

HE-24 bound all the BG-A tetrasaccharide variants with similar ability. Interestingly, it displayed very strong density-dependent binding effects. In most cases, binding to the high density variant was about 100-fold stronger than binding to the corresponding low density variant. Binding to the BG-A trisaccharide was similar to the various tetrasaccharides, but there was no binding to GalNAcα1–3Gal even at a dilution of 1:25. Binding to glycopeptides containing GalNAcα-Ser was slightly better than binding to the BG-A tetrasaccharides, but there were no signals for any glycopeptides containing GalNAcα-Thr, even at a dilution of 1:25.

Discussion

In this study, we evaluated binding properties of eight monoclonal antibodies to the BG-A antigen. Our study focused on recognition in the context of multivalent binding, which is most relevant to common applications of these antibodies. The results provide new insights into the binding properties of these antibodies.

Glycan size and carrier chain had a major influence on antibody binding. Selective recognition of BG-A on certain carrier chains is

well known. One rationale is that monosaccharide residues beyond the BG-A trisaccharide provide additional contacts with the antibody and/or influence BG-A conformations to enhance binding. In these cases, smaller substructures of the optimal determinant have weak binding due to fewer contacts or conformational constraints. Our data highlight a second situation wherein a di- or trisaccharide provides all the necessary contacts to achieve tight binding, and the carrier chain is either tolerated or not tolerated by the antibody. For example, two antibodies (i.e. Z2A and 87-G) bound similarly to GalNAcα1–3Gal, the BG-A trisaccharide, BG-A2 and BG-A6; however these antibodies had markedly reduced binding to BG-A displayed on type 1, 3, 4 and 5 chains. The influence of a reducing end sugar can extend to more remote positions away from the terminal recognition domain. For example, Z2A bound BG-A1 but not the hexasaccharide A-LeB, which contains BG-A1 as its terminal tetrasaccharide. Additionally, 9A bound BG-A4 but not Globo A, which contains BG-A4 as its terminal tetrasaccharide (see Table II and Supplementary data, Figure S1). Although A-LeB and Globo A have the appropriate tetrasaccharide determinants for binding to Z2A and 9A, respectively, the additional monosaccharide residues appended to the tetrasaccharides block recognition by these antibodies in our experimental system. Cases where the carrier chain acts as a "selectivity filter" have been observed in other systems, such as monoclonal antibodies to bacterial glycan determinants (Liu et al. 2015) and fungal glycan determinants (Johnson et al. 2012). Moreover, many human serum antibodies will bind short oligosaccharides alone but not when displayed as part of a larger glycan (Bovin et al. 2012).

Glycan density significantly influenced recognition for some antibodies. For example, HE-24 and 9A only bound glycans at high density. Differences in density-dependent binding properties observed on the array are consistent with known agglutination properties. A1 and A1B red blood cells are known to have a much higher density of BG-A epitopes on their surface than A2 or A2B (Cartron et al. 1974). HE-24, which only bound high density glycans, only agglutinates A1 and A1B red blood cells (Némec et al. 1987). HE-195, which bound both high and low density glycans on the array, agglutinates A1, A1B, A2 and A2B red blood cells (Némec et al. 1987). Given the size of the density effects and their unpredictable nature, variations in presentation are useful for assessing antibody specificity.

Recognition of non-BG-A glycans is also a critical consideration. Potential binding of BG-B and BG-H has been studied previously for most BG-A antibodies, but much less is known about reactivity with non-ABH glycans. Antibody 9A reacted strongly with Tn glycopeptides and the Forssman antigen. The Tn antigen is a tumor-associated carbohydrate antigen and the Forssman antigen is a glycolipid xenoantigen. Thus, binding of 9A to a biological sample could be due to a glycan other than BG-A. HE-24 also reacted with the Tn antigen but only when the GalNAc was attached to the side chain of a serine residue. Like 9A, binding of HE-24 in a test sample could be due to expression of the Tn antigen rather than BG-A. Moreover, this result highlights the importance of evaluating potential reactivity with both serine and threonine variants of the Tn antigen. Reactivity of BG-A antibodies with Tn appears to be relevant to serum antibodies, too. Treatment of human serum with a BG-A trisaccharide resin significantly decreased serum antibody binding to Tn glycopeptides (Campbell et al. 2014).

Finally, small oligosaccharides are often considered insufficient to fully define recognition properties of glycan-binding antibodies and lectins (Lowary 2013). This view primarily stems from cases where small oligosaccharides do not provide sufficient contacts or conformational structure to achieve tight binding. Our results highlight a second and perhaps less appreciated situation wherein small oligosaccharides bind well but do not provide adequate structural information to understand specificity. For example, recognition of the BG-A trisaccharide alone did not indicate whether an antibody would bind larger variants of BG-A, such as BG-A1 or A-LeB. Thus, variations in glycan size and presentation are crucial for fully assessing both affinity and specificity of glycan-binding antibodies.

Materials and methods

Array fabrication and binding assay

Array fabrication is described in the Supplementary data. Type 1–6 A, B and H blood group antigens were generously provided by Prof. Todd Lowary (University of Alberta, Canada) (Meloncelli and Lowary 2009, 2010; Meloncelli et al. 2011; Zhang et al. 2015). BG-A1 and BG-A2 with a different linker ("Sp") were generously provided by the CFG (The Scripps Research Institute, San Diego, CA). The array platform and assay have been described previously (Manimala et al. 2007; Oyelaran et al. 2009; Campbell et al. 2010). Additional details can be found in the Supplementary data.

Supplementary data

Supplementary data for this article are available online at http://glycob.oxfordjournals.org/.

Funding

This work was supported by the Intramural Research Program of the National Cancer Institute, NIH.

Acknowledgements

We thank the Consortium for Functional Glycomics (GM62116; The Scripps Research Institute), Professor Tom Tolbert (University of Kansas), Professor Lai-Xi Wang (University of Maryland), Professor Xuefei Huang (Michigan State University), Professor Todd Lowary (University of Alberta) and Dr Joseph Barchi (National Cancer Institute) for generously contributing glycans for the array.

Conflict of interest statement

None declared.

Abbreviations

aOSM, asialo-ovine submaxillary mucin; BG-A, blood group A; BG-B, blood group B; BG-H, blood group H; RFU, raw fluorescence units.

References

Arthur CM, Cummings RD, Stowell SR. 2014. Using glycan microarrays to understand immunity. *Curr Opin Chem Biol*. 18:55–61.

Barr K, Korchagina E, Popova I, Bovin N, Henry S. 2015. Monoclonal anti-A activity against the FORS1 (Forssman) antigen. *Transfusion*. 55:129–136.

Barr K, Korchagina E, Ryzhov I, Bovin N, Henry S. 2014. Mapping the fine specificity of ABO monoclonal reagents with A and B type-specific function-spacer-lipid constructs in kodecytes and inkjet printed on paper. *Transfusion*. 54:2477–2484.

Bovin N, Obukhova P, Shilova N, Rapoport E, Popova I, Navakouski M, Unverzagt C, Vuskovic M, Huflejt M. 2012. Repertoire of human natural anti-glycan immunoglobulins. Do we have auto-antibodies? *Biochim Biophys Acta*. 1820:1373–1382.

Campbell CT, Gulley JL, Oyelaran O, Hodge JW, Schlom J, Gildersleeve JC. 2013. Serum antibodies to blood group A predict survival on PROSTVAC-VF. *Clin Cancer Res*. 19:1290–1299.

Campbell CT, Gulley JL, Oyelaran O, Hodge JW, Schlom J, Gildersleeve JC. 2014. Humoral response to a viral glycan correlates with survival on PROSTVAC-VF. *Proc Natl Acad Sci USA*. 111:E1749–E1758.

Campbell CT, Zhang Y, Gildersleeve JC. 2010. Construction and use of glycan microarrays. *Curr Protoc Chem Biol*. 2:37–53.

Cartron JP, Gerbal A, Hughes Jones NC, Salmon C. 1974. 'Weak A' phenotypes. Relationship between red cell agglutinability wand antigen site density. *Immunology*. 27:723–727.

Chen HT, Kabat EA. 1985. Immunochemical studies on blood groups. The combining site specificities of mouse monoclonal hybridoma anti-A and anti-B. *J Biol Chem*. 260:13208–13217.

Clausen H, Hakomori SI. 1989. ABH and related histo-blood group antigens; immunochemical differences in carrier isotypes and their distribution. *Vox Sang*. 56:1–20.

Furukawa K, Clausen H, Hakomori SI, Sakamoto J, Look K, Lundblad A, Mattes MJ, Lloyd KO. 1985. Analysis of the specificity of five murine anti-blood group A monoclonal antibodies, including one that identifies type 3 and type 4 A determinants. *Biochemistry*. 24:7820–7826.

Gooi HC, Hounsell EF, Picard JK, Lowe AD, Voak D, Lennox ES, Feizi T. 1985. Differing reactions of monoclonal anti-A antibodies with oligosaccharides related to blood group A. *J Biol Chem*. 260:13218–13224.

Jeyakanthan M, Tao K, Zou L, Meloncelli PJ, Lowary TL, Suzuki K, Boland D, Larsen I, Burch M, Shaw N, et al. 2015. Chemical basis for qualitative and quantitative differences between ABO blood groups and subgroups: Implications for organ transplantation. *Am J Transplant*. 15:2602–2615.

Johnson MA, Cartmell J, Weisser NE, Woods RJ, Bundle DR. 2012. Molecular recognition of *Candida albicans* (1 → 2)-β-mannan oligosaccharides by a protective monoclonal antibody reveals the immunodominance of internal saccharide residues. *J Biol Chem*. 287:18078–18090.

Lindberg L, Johansson SM, Liu J, Grufman P, Holgersson J. 2011. Is there a clinical need for a diagnostic test allowing detection of chain type-specific anti-A and anti-B? *Transfusion*. 51:494–503.

Lindberg L, Liu J, Gaunitz S, Nilsson A, Johansson T, Karlsson NG, Holgersson J. 2013. Mucin-type fusion proteins with blood group A or B determinants on defined O-glycan core chains produced in glycoengineered Chinese hamster ovary cells and their use as immunoaffinity matrices. *Glycobiology*. 23:720–735.

Lindberg L, Theinert K, Liu J, Holgersson J. 2012. Adsorption of chain type-specific ABO antibodies on Sepharose-linked A and B tetrasaccharides. *Transfusion*. 52:2356–2367.

Liu L, Zha J, Digiandomenico A, McAllister D, Stover CK, Wang Q, Boons GJ. 2015. Synthetic enterobacterial common antigen (ECA) for the development of a universal immunotherapy for drug-resistant enterobacteriaceae. *Angew Chem Int Ed*. 54:10953–10957.

Lowary TL. 2013. Context and complexity: The next big thing in synthetic glycobiology. *Curr Opin Chem Biol*. 17:990–996.

Malomgré W, Neumeister B. 2009. Recent and future trends in blood group typing. *Anal Bioanal Chem*. 393:1443–1451.

Manimala JC, Roach TA, Li Z, Gildersleeve JC. 2007. High-throughput carbohydrate microarray profiling of 27 antibodies demonstrates widespread specificity problems. *Glycobiology*. 17:17C–23C.

Meloncelli PJ, Lowary TL. 2009. Synthesis of ABO histo-blood group type V and VI antigens. *Austr J Chem*. 62:558–574.

Meloncelli PJ, Lowary TL. 2010. Synthesis of ABO histo-blood group type I and II antigens. *Carbohydr Res*. 345:2305–2322.

Meloncelli PJ, West LJ, Lowary TL. 2011. Synthesis and NMR studies on the ABO histo-blood group antigens: Synthesis of type III and IV structures and NMR characterization of type I–VI antigens. *Carbohydr Res*. 346:1406–1426.

Miura T, Sano D, Suenaga A, Yoshimura T, Fuzawa M, Nakagomi T, Nakagomi O, Okabe S. 2013. Histo-blood group antigen-like substances of human enteric bacteria as specific adsorbents for human noroviruses. *J Virol*. 87:9441–9451.

Muthana S, Gulley JL, Hodge JW, Schlom J, Gildersleeve JC. 2015. ABO blood type correlates with survival on prostate cancer vaccine therapy. *Oncotarget*. 6:32244–32256.

Némec M, Drímalová D, Horejsí V, Vanák J, Bártek J, Viklický V. 1987. Murine monoclonal antibodies to human A erythrocytes: Differential reactivity with *N*-acetyl-D-galactosamine. *Vox Sang*. 52:125–128.

Oriol R, Samuelsson BE, Messeter L. 1990. Abo antibodies—Serological behavior and immunochemical characterization. *J Immunogenet*. 17:279–299.

Oyelaran O, McShane LM, Dodd L, Gildersleeve JC. 2009. Profiling human serum antibodies with a carbohydrate antigen microarray. *J Proteome Res*. 8:4301–4310.

Palma AS, Feizi T, Childs RA, Chai W, Liu Y. 2014. The neoglycolipid (NGL)-based oligosaccharide microarray system poised to decipher the meta-glycome. *Curr Opin Chem Biol*. 18:87–94.

Park S, Gildersleeve JC, Blixt O, Shin I. 2013. Carbohydrate microarrays. *Chem Soc Rev*. 42:4310–4326.

Parker PJ, Young S, Gullick WJ, Mayes EL, Bennett P, Waterfield MD. 1984. Monoclonal antibodies against the human epidermal growth factor receptor from A431 cells. Isolation, characterization, and use in the purification of active epidermal growth factor receptor. *J Biol Chem*. 259:9906–9912.

Storry JR, Olsson ML. 2009. The ABO blood group system revisited: A review and update. *Immunohematology*. 25:48–59.

Vanak J, Drimalova D, Smyslova O, Nemec M, Viklicky V, Wisniewski K. 1989. Detection of blood group A antigen expression in human colon cancer using monoclonal antibodies with different specificities. *Neoplasma*. 36:479–488.

Wang L, Cummings R, Smith D, Huflejt M, Campbell C, Gildersleeve JC, Gerlach JQ, Kilcoyne M, Joshi L, Serna S, et al. 2014. Cross-platform comparison of glycan microarray formats. *Glycobiology*. 24:507–517.

Zhang D, Huang P, Zou L, Lowary TL, Tan M, Jiang X. 2015. Tulane virus recognizes the A Type 3 and B histo-blood group antigens. *J Virol*. 89:1419–1427.