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Affinity of monoclonal antibodies for Globo-series glycans

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ABSTRACT

Globo-series glycans are human cell-surface carbohydrates that include stem-cell marker SSEA-4 and cancer-cell antigen Globo H. These two hexasaccharides differ only in their terminal saccharide: *N*-acetylneuraminic acid in SSEA-4 and L-fucose in Globo H. Herein, we evaluated the affinity of the monoclonal antibodies α-SSEA-4 and α-GH for the glycans SSEA-4 and Globo H. Using fluorescence polarization, we find that the two monoclonal antibodies have affinity for their cognate glycan in the low nanomolar range, and have negligible affinity for the non-cognate glycan. Using surface plasmon resonance, we find that each cognate affinity is ~20-fold greater if the glycan is immobilized on a surface rather than free in solution. We conclude that the terminal saccharide plays a dominant role in the ability of monoclonal antibodies to recognize these Globo-series glycans and that the extraordinary specificity of these antibodies supports their use for identifying and sorting stem-cells (α-SSEA-4) and as an agent in cancer immunotherapy (α-GH).

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1. Introduction

1.1. Globo-series glycans

Globo-series glycans comprise a group of neutral glycosphingolipids in which a ceramide is linked to a glycan with a root structure of GalNAcβ3Galα4Galβ4Glc.[1,2] Typically, these glycans are retained on the plasma membrane and cluster into lipid rafts.[3] The endogenous function of this glycan family is largely unknown. Their expression does, however, occur during early stages of development and is thought to mediate cell contact and adhesion.[4] Importantly, changes in these glycans are observed throughout differentiation and during tumorigenesis.[5,6] Two notable hexasaccharide members of this family are stage-specific embryonic antigen-4 (SSEA-4) and Globo H (Fig. 1). These glycans share a common precursor, SSEA-3 (Galβ3GalNAcβ3Galα4Galβ4Glc), but vary in the terminal monosaccharide: β3-linked *N*-acetylneuraminic acid for SSEA-4 and α2-linked L-fucose for Globo H.

1.2. SSEA-4, a stem-cell marker

SSEA-4 was discovered using the monoclonal antibody, MC-813-70 (α-SSEA-4), produced by immunization against human embryonic stem cells.[7] Subsequent analyses found expression of this epitope on many stem cell types as well as induced pluripotent stem cells and embryonic carcinoma cells.[8] Although SSEA-4 expression is not required for stem-cell pluripotency, a decrease in expression is observed upon differentiation.[9] In addition the pentasaccharide precursor, SSEA-3, is also used to identify stem cells and is depleted rapidly from the cell surface upon differentiation. Hence, commercial antibodies for both glycans are often used to identify undifferentiated cells.[10] The use of α-SSEA-3 (MC-613) and α-SSEA-4 enables the identification of spontaneous differentiation and the collection of live stem cells.[11,12] Such live-cell sorting has distinct advantages in stem cell and regenerative therapies,[13] and is not enabled by other known stem-cell markers, such as nuclear transcription factors.[14] More recently, SSEA-4 has been detected on malignant glioma cells,[15] which form the most aggressive and common brain tumors in adults, as well as on breast cancer cells.[16,17] As a result, antibodies against SSEA-4 can illicit complement-dependent cytotoxicity and support the targeting of SSEA-4 in cancer vaccines.

1.3. Globo H, a cancer-cell antigen

Globo H was isolated originally from human breast cancer cell line MCF-7.[18] High-level expression of Globo H has been observed on a variety of other cancer cells, including colon, ovarian, prostate, and lung.[16,19] Identification of this cancer-cell antigen was made possible using the antibody MBr1 (α-GH), which was raised

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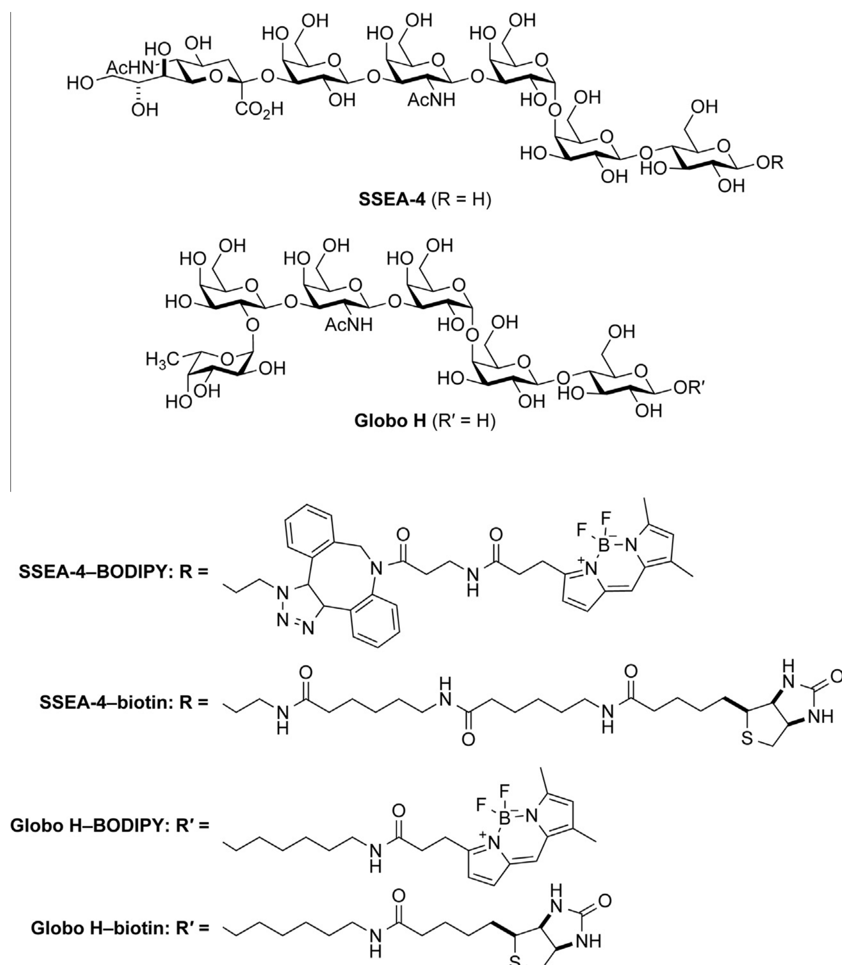

Figure 1. Chemical structures of SSEA-4, Globo H, and their conjugates with BODIPY and biotin.

specifically against MCF-7 cells.[20] Binding assays using printed microarrays demonstrated that α-GH recognizes the terminal tetrasaccharide moiety with 10-fold less affinity than the intact hexasaccharide, and does not bind to the SSEA-3 precursor of Globo H that lacks the terminal L-fucose.[21] Endogenous Globo H expression remains in the apical surface of epithelial tissue, an area somewhat inaccessible to the immune system.[21] As such, Globo H is an attractive target for cancer immunotherapy.[22]

Toward this end, chemical synthesis has been used to access the soluble moiety of Globo H on a large scale.[23] Conjugation of Globo H to other cancer-cell antigens, such as GM2, STn, TF, and KLH, can lead to potential vaccines that induce the production of IgM antibodies that direct the immune system to tumor cells.[17,24,25] Such experimental vaccines are undergoing clinical trials for the treatment of metastatic breast, prostate, lung, and ovarian cancers.[26]

The value of SSEA-4 and its antibody in stem-cell identification and therapies, and of Globo H as an epitope for cancer vaccines is unequivocal. Given the similar structures of SSEA-4 and Globo H (Fig. 1), we sought to determine the specificity of common monoclonal antibodies for each antigen. Investigations of the binding of proteins to cell-surface glycans typically involve printed microarrays, which can provide false-positives and are often less quantitative than other methods.[27] By using synthetic glycan conjugates, fluorescence polarization, and surface plasmon resonance, we provide a quantitative assessment of the affinity of α-SSEA-4 and α-GH for SSEA-4 and Globo H. Our findings provide guidance for a wide range of investigations in biomedicine.

2. Experimental

2.1. Materials

BODIPY-Fl succinimidyl ester was from Invitrogen (Carlsbad, CA). Dibenzocyclooctyne-amine was from Jena Biosciences (Jena, Germany). β-(azidoethyl)SSEA-4 (Compound No B295, Lot S270-1) and SSEA-4–biotin (Compound No B295, Lot S284-1) were provided by the Consortium for Functional Glycomics (San Diego, CA). β-(4-Pentene-1-yl)Globo H was synthesized as described previously.[23,28] α-SSEA-3 IgM monoclonal antibody (MC-613) and α-SSEA-4 IgG3 monoclonal antibody (MC-813-70) were from Thermo Fisher Scientific (Rockford, IL). α-Globo H IgM monoclonal antibody (MBr1) was from Enzo Life Sciences (Farmingdale, NY). Phosphate-buffered saline (PBS; Ca^{2+}- and Mg^{2+}-free) was from Life Technologies (Grand Island, NY). Bovine serum albumin (BSA), biotin, Tween-20, solvents, and other reagents were from Sigma–Aldrich (St. Louis, MO).

2.2. Instrumentation

The identity of synthetic compounds was confirmed by both NMR spectroscopy using a 500 MHz instrument and mass spectrometry using a ULTRAFLEX® III instrument, both from Bruker (Billerica, MA). LC/MS was performed with an LCMS-2020 instrument from Shimadzu (Kyoto, Japan). Fluorescence polarization was recorded on M1000 fluorimeter from Tecan Group

(Mannedorf, Switzerland), and data were analyzed with Prism 5 from GraphPad Software (La Jolla, CA). Surface plasmon resonance (SPR) was measured with a ProteOn XPR 36 System using a NLC NeutrAvidin sensor chip from Bio-Rad Laboratories (Hercules, CA).

2.3. Synthesis of cyclooctyne–BODIPY

We chose BODIPY as our fluorescent probe.[29] This probe is neutral, and its fluorescence is not sensitive to pH. BODIPY-Fl succinimidyl ester (2.5 mg; 6.4 μmol) was dissolved in 0.23 mL of 0.025 M DMF containing dibenzocyclooctyne-amine (1.1 equiv) and triethylamine (3 equiv). Amide-bond formation was monitored by TLC (10% v/v MeOH in DCM). Upon completion of the reaction, the product was purified by preparative TLC to remove remaining reactants and filtration through cotton using MeOH as the solvent to give 3.5 mg (95%) of cyclooctyne–BODIPY. *m*/*z* 568.4 [calc'd for $C_{32}H_{33}BF_2N_5O_2$ (M+NH$_4$) 568.5). ^{1}H NMR (500 MHz, CD_3OD) δ 7.65 (dd, *J* = 7.6, 1.3 Hz, 1H), 7.45 (s, 4H), 7.42 (s, 1H), 7.36 (td, *J* = 7.5, 1.5 Hz, 1H), 7.31 (td, *J* = 7.6, 1.4 Hz, 1H), 7.24 (dd, *J* = 7.5, 1.5 Hz, 1H), 6.96 (d, *J* = 4.1 Hz, 1H), 6.25–6.18 (m, 2H), 5.13 (d, *J* = 14.0 Hz, 1H), 3.70 (d, *J* = 14.0 Hz, 1H), 3.27–3.20 (m, 2H), 3.16–3.07 (m, 2H), 2.50 (s, 3H), 2.47–2.40 (m, 2H), 2.28 (s, 3H), 2.07–1.98 (m, 2H).

2.4. Synthesis of SSEA-4–BODIPY

β-(Azidoethyl)SSEA-4 (0.25 mg; 0.20 μmol) was dissolved in 2.0 mL of 20% v/v H_2O in MeOH. To this solution was added cyclooctyne–BODIPY (5 equiv). The reaction was allowed to proceed overnight. Upon completion of the reaction as monitored by TLC (20% v/v MeOH in DCM), the reaction mixture was concentrated under reduced pressure, and the residue was suspended in H_2O. Free dye was extracted with ether washes to give 0.34 mg (95%) of SSEA-4–BODIPY. LC/MS *m*/*z* 1779 [calc'd for $C_{77}H_{104}BF_2N_9O_{36}$ (M+H) 1781). ^{1}H NMR (500 MHz, 1:4 CD_3OD/D_2O) δ 8.50 (s, 1H), 8.24 (d, *J* = 7.8 Hz, 1H), 8.06–7.91 (m, 1H), 7.72–7.16 (m, 9H), 7.09–6.98 (m, 1H), 6.34–6.24 (m, 2H), 6.09 (d, *J* = 7.5 Hz, 1H), 6.01–5.88 (m, 2H), 5.20–3.09 (m, 47H), 2.85–2.77 (m, 2H), 2.62–2.46 (m, 5H), 2.35–2.26 (m, 3H), 2.08–1.97 (m, 6H), 1.91–1.71 (m, 2H), 1.36–1.23 (m, 2H). ^{19}F NMR (400 MHz, CD_3OD) δ −76.41.

2.5. Synthesis of Globo H–BODIPY

To a solution of β-(4-pentene-1-yl)Globo H (52 mg, 29 μmol) and *N*-*tert*-butyl allylcarbamate (34 mg, 0.22 mmol) in dichloromethane (1 mL) was added the Hoveyda I catalyst (15 mg, 18.2 μmol). The resulting mixture was heated to 37 °C for 2 days and then concentrated under reduced pressure, and the residue was purified by flash chromatography on SiO_2 using a linear gradient of hexane/acetone (2:1 to 1:1) as eluent. Five consecutive short-path columns were necessary to give 24 mg (43%) of the desired olefin metathesis product, *N*-Boc-6-amino-4-hexene-1-yl Globo H, as well as 26 mg of starting material.

To a portion of *N*-Boc-6-amino-4-hexene-1-yl Globo H (24 mg) in MeOH (1 mL) was added 10% w/w Pd on C (10 mg). After purging with H_2(g), a balloon containing H_2(g) was applied to the reaction mixture, which was stirred vigorously for 4 h. H_2(g) was replaced with Ar(g). After filtration through a pre-washed and packed pad of Celite® and concentration, the residue was dissolved in 25% TFA in dichloromethane (1 mL) and stirred for 30 min. The volatiles were removed under reduced pressure and then under high vacuum. The dried residue was dissolved in 1 mL of dichloromethane and successively treated under Ar(g) with DIEA (0.1 mL) and BODIPY–NHS ester (5 mg, 12.8 μmol), and the resulting mixture was stirred in the dark overnight. Solvents were then removed under high vacuum, and the residue was dissolved in MeOH (1 mL), and treated with 20 μL of a solution of NaOMe (25% w/v) in MeOH. Stirring was maintained until LC/MS indicated that the reaction was complete. Neutralization with AcOH and purification by HPLC gave 2 mg of purified product.

LC/MS *m*/*z* 1390 [calc'd for $C_{54}H_{93}N_4O_{32}S$ (M+H) 1390]. ^{1}H NMR (500 MHz, CD_3OD) δ 7.90 (d, *J* = 9.0 Hz, 1H), 7.44 (s, 1H), 7.01 (d, *J* = 3.8 Hz, 1H), 6.32 (d, *J* = 3.8 Hz, 1H), 6.22 (s, 1H), 5.23 (d, *J* = 4.0 Hz, 1H), 4.93 (d, *J* = 4.0 Hz, 1H), 4.55 (3 d, *J* = 7.5 Hz, 3H), 4.42–4.40 (m, 1H), 4.28–4.30 (m, 4H), 4.14–3.64 (m, 27H), 3.57–3.48 (m, 9H), 3.45–3.44 (m, 1H), 3.38–3.15 (m, 27H), 2.59 (t, *J* = 7.7 Hz, 2H), 2.51 (s, 3H), 2.29 (s, 3H), 2.01 (s, 3H), 1.62–1.58 (m, 2H), 1.50–1.46 (m, 2H), 1.42–1.38 (m, 2H), 1.34–1.29 (m, 2H), 1.24 (d, *J* = 6.5 Hz, 3H). ^{19}F NMR (470 MHz, CD_3OD) δ −77.01.

2.6. Synthesis of Globo H–biotin

(*N*-Boc-6-amino-4-hexene-1-yl)Globo H (42 mg) was dissolved in 1.5 mL of 25% v/v TFA in dichloromethane, and the reaction mixture was stirred for 30 min. Volatiles were removed under reduced pressure, and the residue (42 mg) was used directly in the next step. To a solution of biotin (34 mg, 0.14 mmol) in 0.5 mL NMP were added HATU (52 mg, 0.14 mmol) and 0.3 mL of DIEA. After stirring for 5 min, the deprotected amino alkyl Globo H (42 mg) in 1.5 mL DMF was introduced through a syringe. Stirring overnight, concentration under reduced pressure (cold water bath), and purification by flash chromatography using a linear gradient of MeOH/dichloromethane (2–6% v/v) gave 42 mg of crude β-(6-biotinamido-4-hexene-1-yl)Globo H.

The previous product (42 mg) was dissolved in MeOH (1 mL), and to this solution was added 10% w/w Pd on C (10 mg). After purging with H_2(g), a balloon containing H_2(g) was applied to the reaction mixture, which was stirred vigorously for 2 h. H_2(g) was replaced with Ar(g), and the reaction mixture was filtered through a pre-washed and packed pad of Celite®. After concentration of the filtrate, the residue was purified by flash chromatography using a linear gradient of MeOH/DCM (1–6%) to afford pure protected β-(6-biotinamido-1-hexyloxy)Globo H (38 mg). To this peracetylated biotinamidoalkyl Globo H (38 mg) in 1.2 mL MeOH was added 35 μL of NaOMe (25% w/v) in MeOH, and the resulting solution was stirred until analysis by LC/MS indicated that the deprotection was complete (~4 h). The pH was then brought carefully to neutrality with AcOH, and the product was concentrated under reduced pressure and purified by chromatography on Bio-Gel P-4 Gel from Bio-Rad Laboratories using water as eluent, and lyophilized to give 20.3 mg of β-(6-biotinamido-1-hexyl)Globo H. (LC/MS *m*/*z* 1342 [calc'd for $C_{54}H_{93}N_4O_{32}S$ (M+H) 1342]. ^{1}H NMR (500 MHz, D_2O), δ 5.15 (d, *J* = 3.5 Hz, 1H), 4.82 (d, *J* = 3.1 Hz, 1H), 4.56–4.52 (m, 2H), 4.48–4.39 (m, 3H), 4.36–4.30 (m, 2H), 4.17–4.13 (m, 2H), 4.03 (bs, 1H), 3.96–3.46 (m, 32H), 3.27–3.20 (m, 2H), 3.13–3.08 (m, 2H), 2.92 (dd, *J* = 4.9 Hz, *J* = 13 Hz, 1H), 2.85 (m, 3H), 2.71 (d, *J* = 13 Hz, 1H), 2.19–2.16 (m, 1H), 1.97 (bs, 2H), 1.66–1.13 (m, 16H).

2.7. Fluorescence polarization binding assay

The affinity of antibodies for glycans was quantified by monitoring the fluorescence polarization of SSEA-4–BODIPY and Globo H–BODIPY upon addition of α-SSEA-4 and α-GH antibodies. Measurements were performed on 100-μL solutions in the wells of a 96-well plate containing glycan (25 nM) and BSA (7.5 μg) in PBS, pH 7.3. In addition, the affinity of BSA for glycans was determined by monitoring the fluorescence polarization upon addition of BSA. After 30 min at 25 °C, polarization was recorded and values of the equilibrium dissociation constant (K_d) were determined by fitting the data with non-linear regression analysis to the equation:

$$P = \frac{\Delta P[\mathrm{Ab}]^h}{(k_d^n + [\mathrm{Ab}]^h)} + P_{\min} \quad (1)$$

where P is the average of the measured polarization values, ΔP (=$P_{\max} - P_{\min}$) is the difference in anisotropy values of bound and free glycan, [Ab] is the total concentration of antibody, and h is the Hill coefficient.

2.8. Surface plasmon resonance binding assay

The affinity of antibodies for glycans was also quantified by monitoring the SPR as α-SSEA-4 and α-GH were flowed over Globo H–biotin and SSEA-4–biotin bound to a NeutrAvidin chip. The chip was conditioned with 30-μL injections of 50 mM NaOH and 1.0 M NaCl at a flow rate of 30 μL/min in both vertical and horizontal paths. Running buffer was PBS, pH 7.3, containing BSA (0.1 % w/v) and Tween-20 (0.005% v/v), and the chip surface was maintained at 25 °C. The surface was labeled in the vertical channel with SSEA-4–biotin or Globo H–biotin at 0.5 μg/mL with a 300-s injection at 30 μL/min. Binding to the chip surface led to an increase of 40–100 RU. One lane was labeled with 0.5 μg/mL biotin. The chip was rotated in the horizontal direction and stabilized with a 30-μL pulse of 1.0 M NaCl at a flow rate of 30 μL/min, followed by 3 pulses of 30 μL buffer at 100 μL/min. Analyte (antibody or buffer) was applied at various concentrations across the horizontal path at 100 μL/min with a dissociation time of 750 s. The surface was regenerated with 30 μL of 0.10 M glycine, pH 1.7, at a flow rate of 30 μL/min. Equilibrium binding isotherms for the binding of α-SSEA-4 and α-GH to their respective glycans were determined by plotting the response unit at equilibrium (RU_{eq}) versus antibody concentration in GraphPad, and fitting the data to Eq. 1. Sensorgrams for the binding of α-SSEA-3 were analyzed with ProteOn software using a kinetic bivalent fit, and values of K_d were calculated by fitting the kinetic data from eight experiments using four different antibody concentrations. All SPR data were assessed for goodness-of-fit using the criteria that the χ^2 values lie between 10% RU_{max} and 10% RU − χ^2.

3. Results and discussion

Two quantitative solution-based assays were used to characterize the affinity between important human cell-surface glycans and relevant monoclonal antibodies. Fluorescence polarization was used to assess binding in solution, and SPR was used to assess binding on a surface.

3.1. Affinity of monoclonal antibodies to Globo-series glycans in solution

We used the fluorescence polarization of BODIPY-labeled SSEA-4 and Globo H to monitor binding to a monoclonal antibody, α-SSEA-4. We found that the antibody exhibited high affinity to soluble SSEA-4–BODIPY with K_d = (115 ± 10) nM, h = 1.9 in PBS (Fig. 2A). [If the Hill coefficient were fixed at h = 2.0, then K_d = (112 ± 6) nM.] We assessed further any non-specific affinity of α-SSEA-4 toward Globo H, which shares five of the six saccharides of SSEA-4. We found that α-SSEA-4 interacts only weakly with Globo H–BODIPY even at nearly micromolar concentrations, indicating that the antibody distinguishes markedly between the terminal *N*-acetylneuraminic acid of α-SSEA-4 and L-fucose of Globo H.

We also assayed the affinity of Globo H–BODIPY to its respective monoclonal antibody, α-GH. We were able to determine a value of K_d = (161 ± 13) nM, h = 4.8 in PBS (Fig. 2B). [If the Hill coefficient were fixed at h = 2.0, then K_d = (322 ± 88) nM.] In analogy to α-SSEA-4, the affinity of the α-GH antibody was specific for Globo H over SSEA-4.

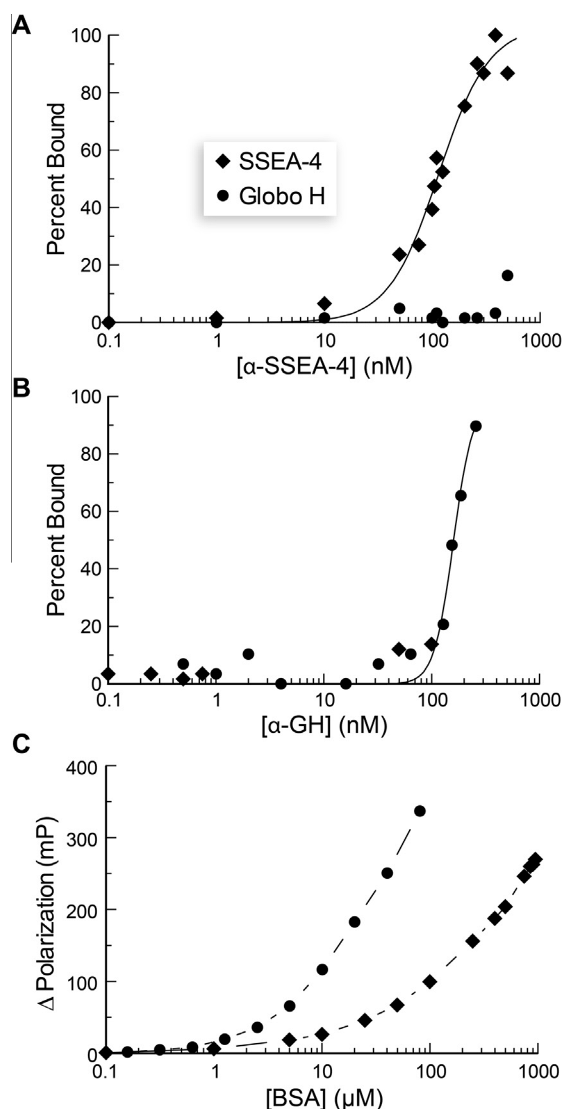

Figure 2. Representative binding isotherms of monoclonal antibodies to glycans in solution as determined with fluorescence polarization. SSEA-4–BODIPY and Globo H–BODIPY were incubated with increasing concentrations of (A) α-SSEA-4 or (B) α-GH in PBS containing BSA, or (C) BSA in PBS. Polarization was normalized to determine the percent bound at each concentration. Data were analyzed by nonlinear regression to Eq. 1 (n = 3) to give K_d = (115 ± 10) nM for the α-SSEA-4·SSEA-4–BODIPY complex and K_d = (161 ± 13) nM for the α-GH·Globo H–BODIPY complex.

Finally, we assessed the affinity of SSEA-4–BODIPY and Globo H–BODIPY for BSA. Both glycans demonstrated only weak affinity for BSA (Fig. 2C) and binding did not achieve saturation. The non-specific binding of Globo H to BSA did appear to be stronger than that of SSEA-4, consistent with the preference of BSA for hydrophobic moieties[30] and the L-fucose of Globo H being more hydrophobic than the *N*-acetylneuraminic acid of SSEA-4.

3.2. Affinity of monoclonal antibodies to Globo H and SSEA-4 on a surface

Next, we used a highly sensitive assay based on SPR to measure the affinity of SSEA-4 and Globo H for α-SSEA-4, α-GH, and α-SSEA-3 antibodies. Using a Neutravidin chip and biotinylated SSEA-4 and Globo H, we were able to create two-dimensional surfaces of the

glycans. Flowing an antibody over that surface enabled us to monitor binding from the increase in response units (RU).

We found that α-GH and α-SSEA-4 bound specifically to their respective hexasaccharide antigen (Fig. 3A and B). In addition, we found that α-SSEA-3 bound more tightly to SSEA-4 than to Globo H (Fig. 3C). Plotting RU at saturation versus concentration yielded equilibrium binding isotherms (Fig. 4A and B). Fitting these data to Eq. 1 provided K_d values of (7.5 ± 0.4) nM, $h = 1.7$ for the α-SSEA-4·SSEA-4 complex and (8 ± 3) nM, $h = 1.5$ for the α-GH·Globo H complex. [If the Hill coefficients were fixed at $h = 2.0$, then the values of K_d would be (7.3 ± 0.3) nM and (7 ± 2) nM, respectively.]

The measured affinity of the monoclonal antibodies for glycans displayed on a surface exceeds that for soluble glycans by ~20-fold. This discrepancy is likely due to multivalency.[31–34] The antibodies

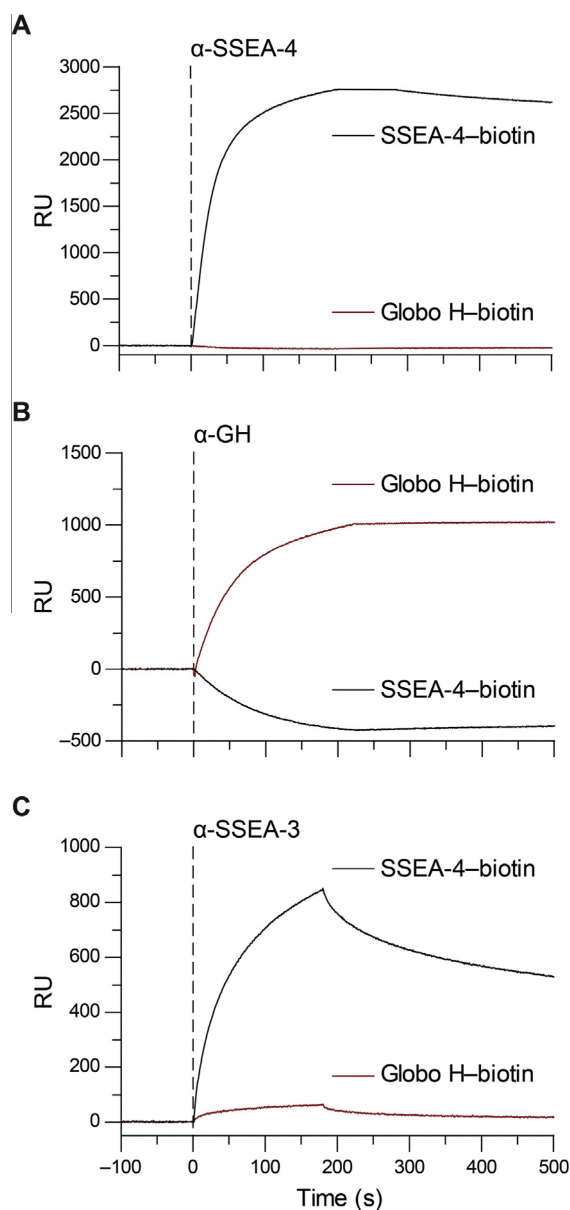

Figure 3. Specificity of monoclonal antibodies for glycans on a surface as determined with SPR. Representative SPR sensorgrams of monoclonal antibodies (A) α-SSEA-4, (B) α-GH, or (C) α-SSEA-3 interacting with Globo H (red) or SSEA-4 (black) immobilized on a NeutrAvidin chip. Antibodies were flowed over the horizontal channels. Curves were referenced to interspots on the horizontal channel to correct for non-specific interactions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

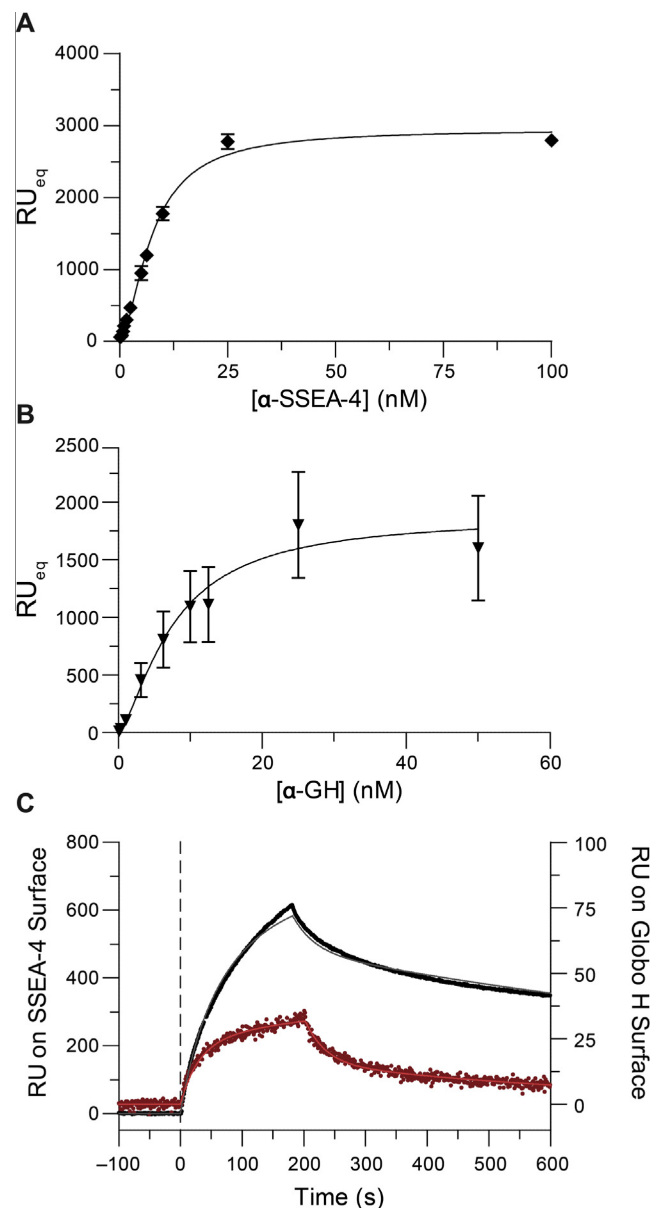

Figure 4. Affinity of monoclonal antibodies for glycans on a surface as determined with SPR. Response units at saturation (RU_{eq}) were recorded for increasing antibody concentrations and (A) SSEA-4 ($K_d = 7.5 \pm 0.4$ nM) and (B) Globo H ($K_d = 7 \pm 4$ nM) surfaces. Data were analyzed by non-linear regression to Eq. 1. (C) Sensorgram of α-SSEA-3 binding to SSEA-4 (black; $K_d = 18 \pm 2$ nM) and Globo H (red; $K_d = 0.5 \pm 0.2$ μM) surfaces. Data were fitted to a kinetic bivalent binding curve (thin line) with ProteOn software. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

are bivalent, and can bind to two surface-displayed glycans simultaneously with a concomitant increase in affinity. This arrangement could better mimic binding to the surface of a human cell.[33,34]

SSEA-3, while mainly a stem cell marker, has been observed to be overexpressed on teratocarcinoma cells.[7,35] Due to the weaker affinity of α-SSEA-3 for the glycan surfaces, we were able to fit the sensorgrams using a kinetic bivalent fit to each curve (Fig. 4C), to give K_d values of (18 ± 2) nM for the α-SSEA-3·SSEA-4 complex and (0.5 ± 0.2) μM for the α-SSEA-3·Globo H complex. Thus, α-SSEA-3 has a significant preference for the *N*-acetylneuraminic acid of SSEA-4 than the L-fucose of Globo H.

Prior assessments of the affinity of monoclonal antibodies to glycan antigens used microarray technology. In that way, α-SSEA-4 had been shown to form a complex with SSEA-4 having

a K_d value of (4.21 ± 0.26) nM,[15] and α-GH had been shown to form a complex with Globo H having a K_d value of (0.56 ± 0.129) nM.[21] Our data are consistent with this low nanomolar affinity for surface-displayed glycans, and reveal a high specificity.

4. Conclusions

We conclude that the monoclonal antibodies α-SSEA-4 and α-GH bind specifically to their cognate antigens with affinity in the low nanomolar range, both in solution as well as on a two-dimensional surface. Moreover, the antibodies have virtually no cross-reactivity for the non-cognate glycan. These data support the reliability and utility of these monoclonal antibodies for the identification of stem cells and for applications in cancer immunotherapy.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2014.07.003.

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