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Analysis of the Specificity of Five Murine Anti-Blood Group A Monoclonal Antibodies, Including One That Identifies Type 3 and Type 4 A Determinants†

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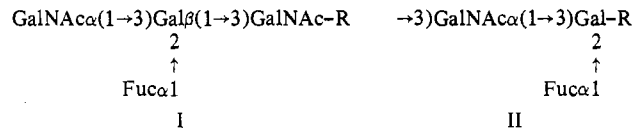
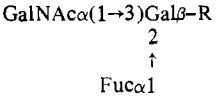
ABSTRACT: The specificity of five mouse monoclonal anti-A blood group antibodies (Ab), four of which were produced by immunization with cultured human cancer cells and one with a synthetic antigen, has been determined by examining their reactivity with purified A glycolipids, erythrocyte glycolipids, oligosaccharides, ovarian cyst glycoproteins, and salivary glycoproteins. Two of the antibodies (HT29-36 and CB) reacted with all A variant structures tested and have a broad anti-A reactivity. Ab CLH6 did not agglutinate A erythrocytes and reacted preferentially with the type 1A structure. Ab S12 agglutinated all A_1 erythrocytes and reacted best with simple, monofucosyl type 2 A structures, such as A^a-2, A^b-2, and A tetrasaccharide. Ab M2 has a novel, but complex, spectrum of reactivity. It reacts with type 3 and type 4 A chains and not with type 1 and type 2 A chains. It appears to recognize both an external A structure



(I) (found) in type 3 and type 4 chains) and also an internal structure (II) found in type 3 chains. Ab M2 agglutinates all A and AB erythrocytes but does not react with salivary glycoproteins.

Although Landsteiner originally described A, B, and O blood groups on the surface of erythrocytes, it has long been known that these antigens are not confined to red cells but are found in most secretions and in many tissues of the human body (Race & Sanger, 1975). The immunodominant structure for the blood group A determinant, which is one of the most extensively studied specificities, is considered to be



However, it has been found that the remainder of the carbohydrate chain (R) has much influence on the precise specificity of the determinant. Different blood group A variant structures have been described that have different sugars and sugar sequences in the R region (Kabat, 1973; Watkins, 1980; Hakomori, 1981). For example, the A determinants (as well as the B, H, and Lewis determinants) are based on two different carbohydrate sequences, i.e., Galβ(1→3)GlcNAcβ-(1→3)Galβ- or Galβ(1→4)GlcNAcβ(1→3)Galβ-. These structures are designated type 1 and type 2 chains, respectively. More recently, a type 3 chain blood group determinant [extended or repetitive A: GalNAcα(1→3)Galβ(1→3)-GalNAcα(1→3)Galβ(1→4)GlcNAc] and a type 4 A chain based on the globo sequence [GalNAcα(1→3)Galβ(1→3)-GalNAcβ(1→3)Galα(1→4)Gal] have been described (Clausen et al., 1984, 1985).

Following the development of the hybridoma technique, a number of anti-blood group A monoclonal antibodies have

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been produced (Barnstable et al., 1978; Voak et al., 1980; Edelman et al., 1981; Messeter et al., 1984; Abe et al., 1984). For only a few of these, however, have the antigenic determinants recognized been precisely determined. In this study we have elucidated the specificity of five murine anti-A monoclonal antibodies, including one that identifies type 3 and type 4 determinants.

Materials and Methods

Antibodies. The origin and properties of the moAbs[1] used in this study are summarized in Table I. Ab M2 was produced by immunization of mice with the renal cancer cell line SK-RC-28 and has been described (Ueda et al., 1981). Ab HT29-36 and Ab CLH6 were produced by immunization with the colonic cancer cell line HT29, and Ab S12 resulted from immunization with the ovarian cancer cell line SK-OV-4; hybridomas were produced by a procedure already described (Ueda et al., 1981). Ab CB is a commercial anti-A typing serum produced by the Chembiomed Co. (Edmonton, Alberta, Canada).

Glycolipids. The structures of the blood group A active glycolipids and a few of their precursor derivatives used in this study are shown in Figure 1. Type 1 A^a (A^a-1) was isolated from human gastric cancer cell line MKN45 as described by Abe et al. (1984). Type 2 A^a (A^a-2) and type 2 A^b (A^b-2) were extracted from human A erythrocytes and were purified and identified as described by Hakomori et al. (1972). Type 3 A (A^a-3) and A^x (type 4 chain A or globo-A) are recently described human red cell glycolipids (Clausen et al., 1984, 1985). Type 3 chain (H_1-3) and β-galactosyl-A were prepared according to a method to be described elsewhere (H. Clausen, S. B. Levery, and S. Hakomori, unpublished results).

Ceramide trihexoside, globoside, GM1, GD1a, and GT were obtained from Supelco. Other gangliosides were isolated from human melanoma cells as described previously (Pukel et al., 1982). Lacto-*N*-tetraosyl- and lacto-*N*-neotetraosylceramides and Le^a-, Le^b-, H-1-, H-2-, X-, and Y-active glycolipids were kindly provided by Drs. Breimer and Karlsson and have been described (McKibbin et al., 1982; Lloyd et al., 1983). Forssman antigen [GalNAcα(1→3)GalNAcβ(1→3)Galα-(1→4)Galβ(1→4)Glc-Cer] was a gift from Dr. D. M. Marcus (Baylor College of Medicine, Houston, TX).

Glycoproteins and Oligosaccharides. Human A (MSS 10%, Sullivan), B (Beach, phenol insoluble), HLe^b (Tighe, phenol insoluble), Le^a (N-1, 10% of 20%), and precursor (OG, 10% of 20%) ovarian cyst fluid glycoproteins and hog gastric mucin were kindly provided by Dr. E. A. Kabat (Columbia University, New York, NY) and have been described (Lloyd & Kabat, 1968). Salivary glycoproteins were prepared from saliva by Pronase digestion as described by Sakamoto et al. (1984). Oligosaccharides (A-tetra, A-penta, A-hexa, and A-hepta; Figure 1) were isolated from feces of a breast-fed infant (Sabharwal et al., 1984).

Extraction and Isolation of Glycolipids from Erythrocytes. For the isolation of total neutral glycolipids from A, B, and O erythrocytes, out-dated blood was obtained from the Blood Bank of Memorial Sloan-Kettering Cancer Center. These blood samples were washed twice with ACD solution, and membranes were prepared by hypotonic shock in 7 mM phosphate buffer, pH 7.2. The membrane preparation was

[1] Abbreviations: Ab, antibody; moAb, monoclonal antibody; ELISA, enzyme-linked immunosorbent assay; LNT, lacto-*N*-tetraose; LNneoT, lacto-*N*-neotetraose; PBS, phosphate-buffered saline; TLC, thin-layer chromatography; ACD, acid–citrate–dextrose.

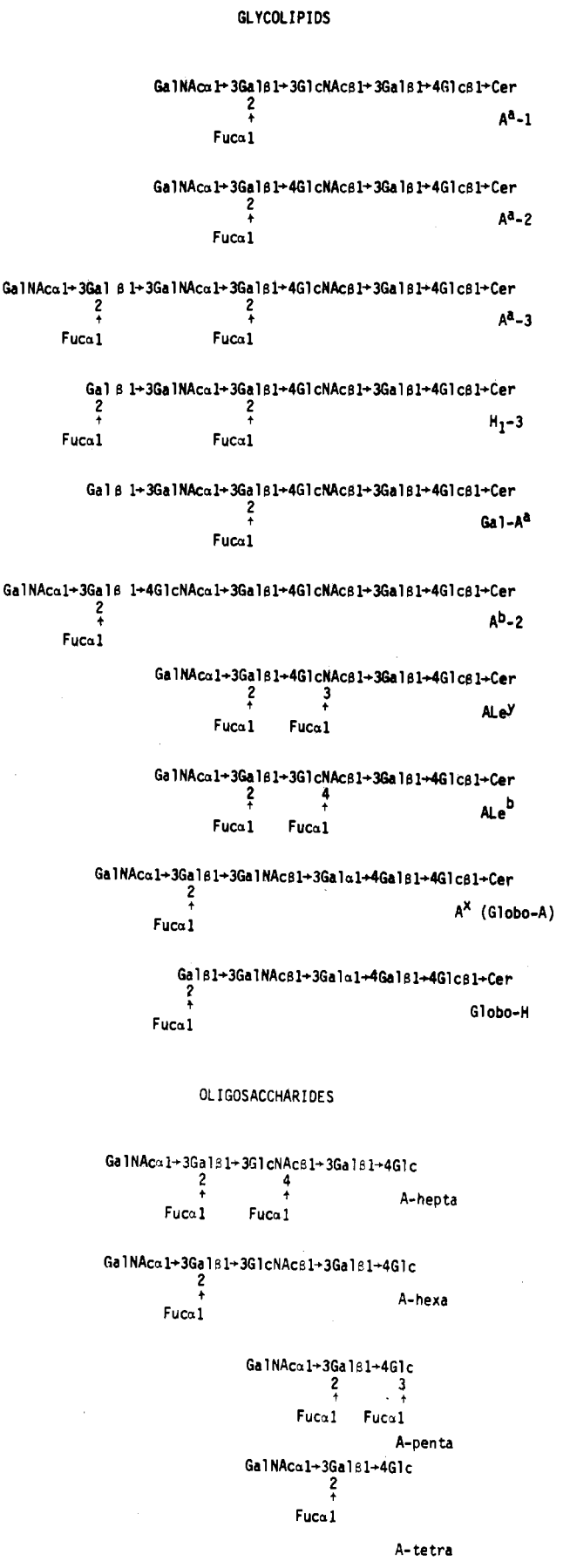

FIGURE 1: Structures of glycolipids and oligosaccharides used in this study.

Table I: Derivation of Monoclonal Antibodies and Agglutination Activity on Erythrocytes

moAb	Ig class	immunizing antigen	agglutination titer[a] A_1	B	O
HT29-36	IgG3	colonic cancer cell line (HT29)	24000	0	0
CB	IgM	synthetic antigen	10000	0	0
CLH6	IgM	colonic cancer cell line (HT29)	0	0	0
S12	IgM	ovarian cancer cell line (SK-OV-4)	16000	0	0
M2	IgM	renal cancer cell line (SK-RC-28)	10000	0	0

[a] Determined by microscopic hemagglutination assay using mouse ascites fluid or serum. Expressed as reciprocal of dilution giving 1+ agglutination.

Table II: Reactivity of Antibodies with Glycoproteins from Ovarian Cysts and Hog Gastric Mucin

moAb[c]	A (MSS)	A (Sullivan)	A+H (hog)	B (Beach)	Le[a] (N-1)	HLe[b] (Tighe)
HT29-36	0.27[a]	0.82	2.5	>1000	>1000	>1000
CB	0.05	0.16	0.48	420	595	707
CLH6	0.03	0.16	12.8	>1000	>1000	>1000
S12	2.5	2.5	9.8	>1000	>1000	>1000
M2	4.3[b]	4.3[b]	1000	>1000	>1000	>1000

[a] Amount of glycoprotein (μg) showing 50% of plateau reaction in ELISA. [b] Partial reaction. [c] Dilutions of antibodies used were as follows: HT29-36, 1:200 of ascites fluid; CB, 1:100 of ascites fluid; CLH6, 1:5 of supernatant culture fluid; S12, 1:5 of supernatant culture fluid; M2, 1:100 of ascites fluid.

lyophilized and extracted sequentially in 30 volumes of 2:1, 1:1, and 1:2 chloroform/methanol. A glycolipid fraction was subsequently isolated by Florosil chromatography of the acetylated sample (Saito & Hakomori, 1971). After deacetylation and desalting, a neutral glycolipid fraction was obtained by DEAE-Sephadex (A-50) chromatography (Yu & Ledeen, 1972).

Hemagglutination Assays. Hemagglutination assays were performed by both macroscopic and microscopic methods. For the macroscopic method, 25 μL of a 1% suspension of erythrocytes in PBS was added to 25 μL of serially diluted antibody in 96-well U-bottom plates (U microtiter plates, Dynotech Laboratories, Inc., Alexandria, VA). The results were read visually after incubation at 4 °C for 2 h. For the microscopic method, 10 μL of 0.1% erythrocyte suspension was added to 10 μL of serially diluted antibody in 60-well HLA plates (Miles Laboratories Inc., Naperville, IL). After being shaken gently, the plates were incubated at 4 °C overnight and examined under a microscope. Agglutination was scored as 4+, 3+, 2+, 1+, or − according to the degree of clumping.

Immunostaining Procedure. The reactivity of antibodies with glycolipids after separation by thin-layer chromatography was performed according to the method of Magnani et al. (1980), with some modification (Young et al., 1983). In brief, the glycolipids were fractionated on aluminum-backed silica gel F254 sheets (Merck, Darmstadt, West Germany) in chloroform/methanol/water (60:35:8). After drying, the sheets were dipped for 1 min in *n*-hexane saturated with poly(isobutyl methacrylate) (Polyscience Inc., Warrington, PA) and then sprayed with PBS containing 2% bovine serum albumin and 0.1% NaN_3, pH 7.3 (solution A). After they were immersed in solution A for 2–3 h, monoclonal antibody solutions were layered on the sheets and incubated for 3 h. After these were washed 5 times with PBS, rabbit anti-mouse IgG (heavy and light chain specific; Cappel Labs, Malvern, PA) was added and incubated for 1.5 h. After a washing, ^{125}I-labeled protein A (Pharmacia Fine Chemicals; 1×10^6 cpm/mL) in solution A was added and incubated for 1 h. After being washed and dried, the sheet was exposed to X-Omat AR film (Eastman Kodak Co., Rochester, NY) for 15–24 h.

Solid-Phase Binding and Inhibition Assays. The reactivity of antibodies with salivary and ovarian cyst glycoproteins was examined by ELISA according to the procedure previously described (Sakamoto et al., 1984). In brief, glycoprotein samples, dissolved in water, were added to the wells of HLA plates, air-dried overnight, and then dried in vacuo over P_2O_5 for 1 h. The plates were then preincubated with PBS containing 0.1% BSA (Sigma Chemical Co., St Louis, MO) for 2 h. Ten microliters of antibody was then added and incubated for 45 min at room temperature. After these were washed 5 times with PBS, 10 μL of goat anti-mouse IgG (whole IgG) conjugated with alkaline phosphatase (Sigma Chemical Co., St Louis, MO) was added at 1:200 dilution. After incubation for 45 min and a washing, 10 μL of *p*-nitrophenyl phosphate (5 mg/mL in diethanolamine; Sigma Chemical Co.) was added. After incubation at 37 °C for 15–30 min, the absorbance was read in an ELISA reader at 405 nm. Inhibition assays were performed by mixing oligosaccharides with an appropriate dilution of the antibody and testing residual antibody reactivity with the ELISA assay using 8 ng of A glycoprotein/well (or neutral A glycolipids, in the case of AbM2).

ELISA was performed on glycolipids according to the same procedure, except that methanol was used for dilution of the glycoplipids and they were dried for 1 h at room temperature [cf. Lloyd et al. (1983)]. Solid-phase radioimmunoassays were performed as described previously (Kannagi et al., 1983).

Results

Reactivity of Monoclonal Antibodies with Erythrocytes. The ability of the five moAbs to agglutinate A, B, AB, and O erythrocytes is shown in Tables I and III. Abs CB, HT29-36, and M2 agglutinated erythrocytes from all A and AB individuals. Ab M2 reacted strongly with red cells from some individuals but weakly with those from other individuals. Ab S12 agglutinated only about 80% of the A and AB samples tested.[2] Ab CLH6 did not agglutinate any of the erythrocytes samples tested. None of the five moAbs agglutinated B or O red cells or sheep red cells.

Reactivity of Monoclonal Antibodies with Glycoproteins. The five monoclonal antibodies were examined for their reactivity with glycoproteins from human ovarian cyst fluids and hog gastric mucin by an ELISA assay. As shown in Table II, all antibodies reacted with MSS and Sullivan A substances except that Ab M2 gave a weaker reaction. Abs HT 29-36

[2] These are all A_1 individuals (Furukawa et al., 1986).

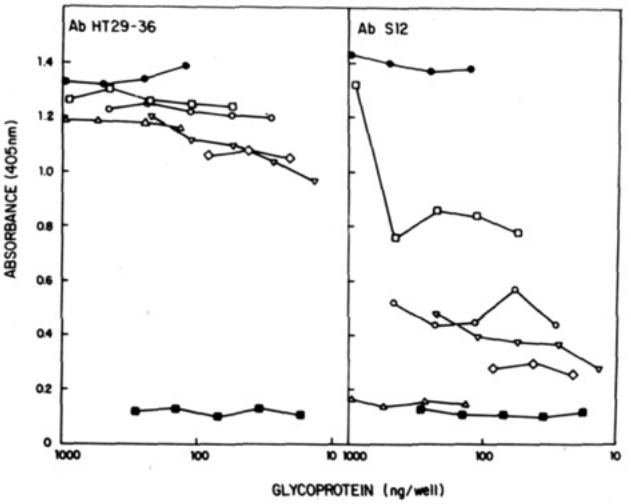

FIGURE 2: Direct binding assays (ELISA) of ovarian cyst glycoprotein and some examples of salivary glycoproteins with Ab HT29-36 and Ab S12. Antibody dilutions: HT29-36, 1:200 of ascites fluid; S12, 1:250 of ascites fluid. Antigens: (●) A ovarian cyst glycoprotein (Sullivan); (□, ○, ▽, ◇) salivary glycoproteins from A secretors; (△) salivary glycoprotein from AB secretor; (■) salivary glycoprotein from A nonsecretor. Since the concentration of glycoproteins differed in the various saliva samples, the amount applied to the first well varied from 90 to 1000 ng.

and CB showed strong reactivity with hog A and H mucins, Abs S12 and CLH6 showed intermediate reactivity, and Ab M2 was unreactive. None of the antibodies showed significant reactivity with B, HLe^b, or Le^a glycoproteins.

Table III: Reactivity of Antibodies with Erythrocytes and with Glycoproteins from Saliva

	erythrocytes[a]			salivary glycoproteins[b]		
moAb	A and AB	B	O	A and AB	B	O
HT29-36	all (32/32)	–	–	all (10/10)	–	–
CB	all (74/74)	–	–	all (10/10)	–	–
CLH6	none (0/32)	–	–	all (9/10)[c]	–	–
S12	some (59/74)[d]	–	–	some (4/10)[e]	–	–
M2	all (74/74)	–	–	none (0/10)	–	–

[a] Hemagglutination tests. [b] ELISA (results for "secretors" only shown); see Figure 2 for exampples. [c] Except for one A_2 individual. [d] See footnote 2. [e] Weak reactivity (see Figure 2).

The reactivity of the antibodies to partially purified salivary glycoproteins from 33 individuals was also tested (Table III). Abs HT29-36, CB, and CLH6 reacted with all A and AB salivas from secretor individuals, except that Ab CLH6 did not react with one A_2 saliva. Ab M2 showed no reaction with any of the saliva samples tested. Ab S12 reacted weakly with saliva from one-third of the A_1 and A_1B individuals; none of the salivas were as reactive as ovarian cyst glycoproteins (Figure 2).

Reactivity of Antibodies with Erythrocyte Total Neutral Glycolipids and Purified A Glycolipids by Immunostaining of Thin-Layer Chromatograms. Abs HT29-36 and CB showed reactivity with two major, one minor, and a few other faint bands in the total neutral glycolipid extracts of A and AB red cells by the immunostaining technique (Figure 3).

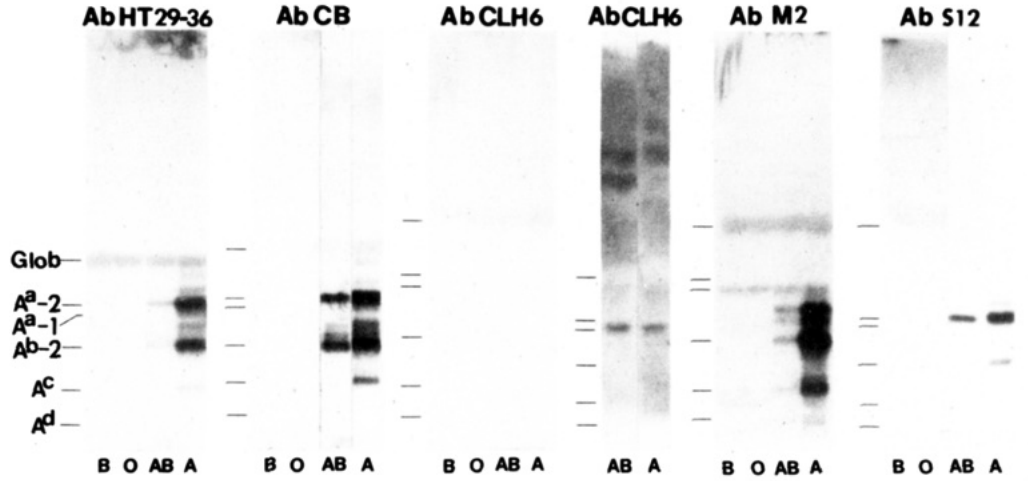

FIGURE 3: Immunostaining of neutral glycolipid fraction from A, AB, B, and O erythrocytes separated by thin-layer chromatography. The migration position of some standard glycolipids are indicated. Since the experiments were not run simultaneously, there was some variation in migration between the different plates; the bars indicate the migration positions of the standard glycolipids in the different runs. Glob: globoside [GalNAcβ(1→3)Galα(1→4)Galβ(1→4)Glc-Cer].

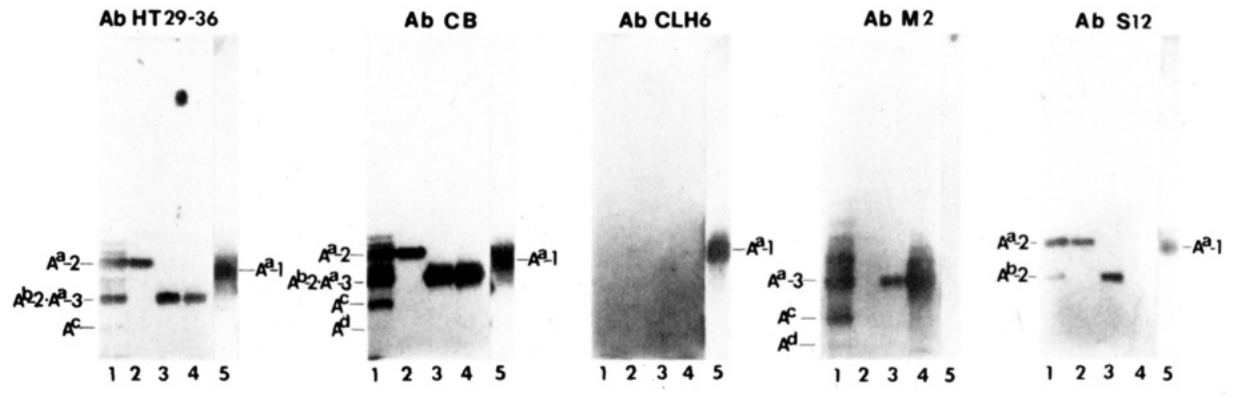

FIGURE 4: Immunostaining of purified glycolipids separated by thin-layer chromatography: (1) A erythrocyte total neutral glycolipid fraction; (2) A^a-2; (3) A^b-2; (4) A^a-3; (5) A^a-1. See Figure 1 for the structures of these glycolipids.

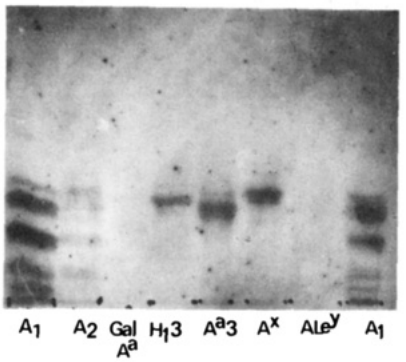

FIGURE 5: Immunostaining of purified glycolipids by Ab M2. See Figure 1 for the structures of these glycolipids.

Table IV: Summary of Reactivity of Antibodies with Glycolipids as Determined by Immunostaining

glycolipid	HT29-36	CB	CLH6	S12	M2
A^a-1	++	++	++	+	–
A^a-2	++	++	–	++	–
A^a-3	++	++	–	–	++
A^b-2	++	++	–	++	–
H_1-3	nd	nd	nd	nd	+
A^x	nd	nd	nd	nd	++
Forssman	–	–	–	–	–

Glycolipids from B and O cells did not show reactivity. All samples showed a faint band corresponding to globoside; this was considered to be a nonspecific reaction caused by the preponderence of globoside in these extracts. Using purified glycolipids, it was shown that these two antibodies can react with A^a-1, A^a-2, A^b-2, and A^a-3 determinants (Figure 4). On the basis of this information and their migration rates, the two major A red cell glycolipids reacting with Abs HT29-36 and CB (Figure 3) can be identified as A^a-2 (upper band) and A^b-2 and A^a-3 (lower band).

Ab CLH6 was unreactive with glycolipids extracted from A, AB, B, and O red cells under the standard autoradiogram exposure times (Figure 3). With longer exposures, Ab CLH6 could be shown to react weakly with a component in A and AB cells migrating just slower than A^a-2 (Figure 3, fourth panel); this position corresponds to the migration position of A^a-1. In fact, when Ab CLH6 was reacted with the standard glycolipids, it was found that only A^a-1 glycolipid was stained with this antibody (Figure 4).

Ab S12 reacted with only two components in glycolipids extracted from A and AB erythrocytes and did not react with O or B cell glycolipids (Figure 3). Analysis with purified glycolipids showed that Ab S12 reacted with A^a-2 and A^b-2 and weakly with A^a-1 (Figure 4). Ab S12 was unreactive with A^a-3 glycolipid. On the basis of these results, it is apparent that Ab S12 stains only A^a-2 and A^b-2 in red cell glycolipids and is unreactive with any other species in these extracts.

Ab M2 identified numerous glycolipids in extracts of A and AB erythrocytes but not from extracts of B or O erythrocytes (Figure 3). This antibody reacted strongly with type 3 chain A (A^a-3) but not with A^a-2, A^b-2, or A^a-1 in immunostaining of thin-layer plates (Figure 4); the band observed with the A^b-2 sample is probably due to reactivity with A^a-3, which is known to contaminate this sample. Ab M2 also reacted with A^x and H_1-3 glycolipids but not with Gal-A^a or ALe^b in immunostaining (Figure 5). These results were confirmed by a solid-phase radioimmunoassay that showed strong reactivity of Ab M2 with A^a-3 and A^x, moderate reactivity with H_1-3, and weak reactivity with A^a-2, A^a-1, and Gal-A^a; globo-H, ALe^y, and ALe^b were unreactive in this assay (Figure 6).

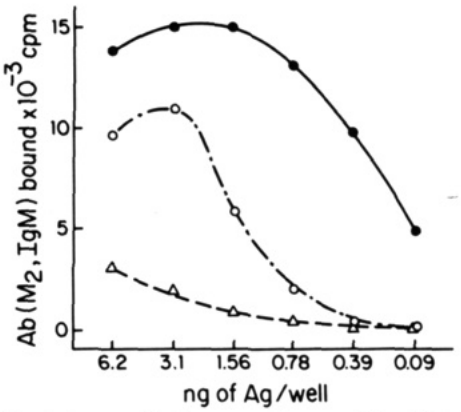

FIGURE 6: Radioimmunobinding assay for reactivity of Ab M2 with purified glycolipids. Antibody dilution was 1:500 of ascites fluid. (●) A^x and A^a-3; (○) H_1-3; (△) A^a-2, A^a-1, and Gal-A^a. No reactivity was found with globo-H, ALe^y, or ALe^b. See Figure 1 for the structures of these glycolipids.

Table V: Inhibition of Monoclonal Antibodies by Oligosaccharides[a]

	oligosaccharide			
antibody[b]	A-tetra[c]	A-penta	A-hexa	A-hepta
HT29-36	1.3[d]	0.9	1.9	0.7
CB	5.8	4.0	6.1	2.2
CLH6	14.1	42.6	0.2	1.0
S12	3.0	12.2	10.7	24.3
M2	>52.6	>42.6	>34.5	24.3

[a] Determined by ELISA method as described under Materials and Methods. [b] Abs (10 μL) were diluted as follows: HT29-36 (1:80); CB (1:75); CLH6 (undiluted culture fluid); S12 (1:200); M2 (undiluted culture fluid). [c] See Figure 1 for structure of oligosaccharides. [d] Nanomoles required for 50% inhibition.

The results of these immunostaining experiments and the lack of reactivity of the antibodies with Forssman antigen are summarized in Table IV. None of the antibodies reacted with a number of other neutral and acidic glycolipids tested (CTH, globoside, LNT, LNneoT, H-1, H-2, Le^a, Le^b, X, Y, GM2, GM3, GM1, GD1a, GD2, and GT).

Oligosaccharide Inhibition Assays. The results obtained by inhibition of the antibody reaction with four A-active oligosaccharides were generally in agreement with those obtained with glycolipid binding assays (Table V). Thus, Abs HT29-36 and CB were inhibited by both type 1 and type 2 structures and by mono- and difucosyl oligosaccharides, confirming the broad specificity of these antibodies. Ab CLH6 was inhibited most strongly by the two type 1 chain oligosaccharides (A-hexa and A-hepta) whereas with Ab S12 the simplest type 2 structure (A-tetra) was the best inhibitor. Ab M2 was not inhibited by the four oligosaccharides at the concentration tested, in agreement with its specificity for type 3 and 4 chains.

Discussion

Human blood group antigens have traditionally been detected with allogeneic sera that contain polyclonal antibodies with heterogeneous specificities. Recently, monoclonal antibodies to blood group antigen have been produced some of which are suitable for use as typing reagents (Voak et al., 1980; Messeter et al., 1984). One of the most important properties of monoclonal antibodies is their capability to discriminate between fine differences of antigenic structures. It is therefore important to define the precise specificity of monoclonal antibodies as they may discriminate between antigenic determinants in a manner not possible with conventional antisera. In this study we have elucidated the specificities of five murine anti-blood group A moAbs, four of which were developed by

immunizations with cultured human tumor cells and one with a synthetic antigen.

Abs CB and HT29-36 were derived quite differently but nevertheless showed very similar serological specificity. They both could agglutinate all A and AB erythrocytes tested and reacted, without exception, with all A and AB salivas. In TLC immunostaining they reacted with all blood group A variant structure examined including A^a-1, A^a-2, A^b-2, and A^a-3 (Table IV). In inhibition assays with oligosaccharides they were inhibited by type 1 and type 2 as well as by mono- and difucosyl structures. These results imply that the determinant structure detected by these moAbs was the determinant that is common to all A variants:

$$\begin{array}{l} \text{GalNAc}\alpha(1\rightarrow3)\text{Gal-} \\ \quad\quad\quad\quad\quad\quad 2 \\ \quad\quad\quad\quad\quad\quad \uparrow \\ \quad\quad\quad\quad\quad\quad \text{Fuc}\alpha1 \end{array}$$

In addition to the two main erythrocyte glycolipids (type 2 A^a and type 2 A^b), these two antibodies also stained bands migrating between A^a-2 and A^b-2 on TLC plates. One more band was detected that migrated below A^b-2 and probably corresponds to A^c, and a fainter band moving at a position corresponding to A^d (Hakomori, 1981) was also observed.

Ab CLH6 reacted strongly only with glycolipids and oligosaccharides containing type 1 A determinants, namely

$$\begin{array}{l} \text{GalNAc}\alpha(1\rightarrow3)\text{Gal}\beta(1\rightarrow3)\text{GlcNAc}\beta(1\rightarrow3)\text{Gal-} \\ \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad 2 \\ \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad \uparrow \\ \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad \text{Fuc}\alpha1 \end{array}$$

This antibody did not react with type 2 or type 3 determinants. These results suggest that Ab CLH6 has the same or very similar, specificity as Ab AH21 previously reported by Abe et al. (1984). It is interesting to note that although Ab CLH6 does not agglutinate red cells and reactivity with red cell glycolipids could normally not be detected, longer exposure of the autoradiograms revealed a component migrating with type 1 A^a. This result indicates that although the main glycolipids of red cells are of type 2 and type 3, a small amount of type 1 blood group glycolipids may also be present; these species are probably adsorbed from serum in the same way that Le^a and Le^b are acquired by red cells (Marcus & Cass, 1969).

In contrast to the other antibodies, Ab S12 reacted with only a proportion of red cell samples and salivas from A and AB individuals. In immunostaining experiments, Ab S12 was shown to react only with A^a-2 and A^b-2 (and weakly with A^a-1); it did not recognize any of the longer chain A structures in red cells even on long exposure of the X-ray film. Ab S12 was inhibited best by the A-tetra oligosaccharide. These results indicate that Ab S12 is relatively specific for short, unbranched, type 2 monofucosyl A determinants. The poor reactivity of Ab S12 with A saliva in comparison to ovarian cyst glycoproteins is consistent with the earlier report that salivary glycoproteins are richer in difucosyl than in monofucosyl structures (Sakamoto et al., 1984); the reason why only four out of ten salivas were reactive is unclear.

Ab M2 agglutinated A and AB erythrocytes but did not react with type 2-A^a and 2-A^b glycolipids or with type 1 or type 2 mono- and difucosyl A oligosaccharides. Among eight blood group A variant structures examined, Ab M2 could react with only type 3 glycolipids (A^a-3 and H_1-3) and with type 4 A (A^x) glycolipid. With whole erythrocyte glycolipids, Ab M2 also reacts with components migrating at the positions of A^c and A^d; this result suggests that there may be other red cell glycolipids having type 3 or type 4 structures that have

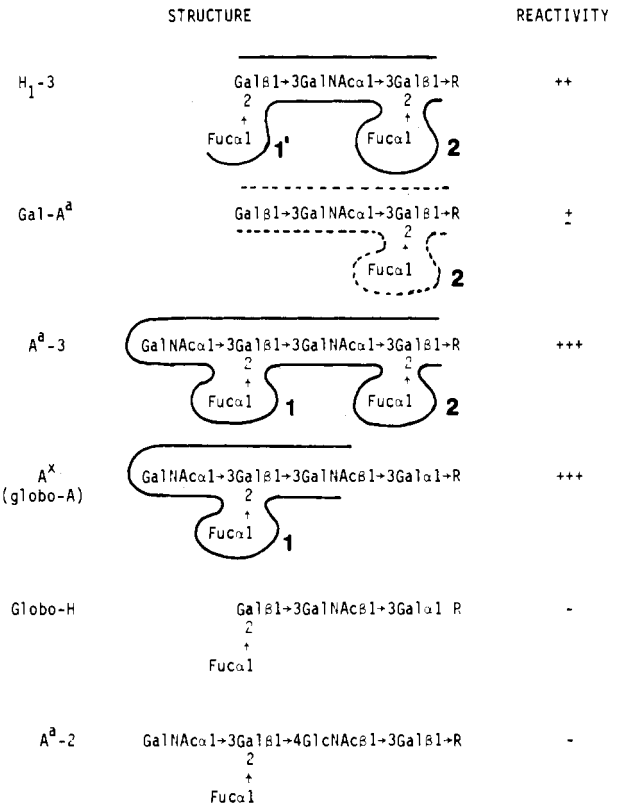

FIGURE 7: Suggested basis for specificity of Ab M2 for type 3 and type 4 determinants showing reactivity with two subsites (1 and 2).

not yet been identified. From these results it is concluded that Ab M2 has a complex specificity. It appears to have a large combining site, comprising two subsites, one recognizing external and the other recognizing internal structures (Figure 7). Since Ab M2 reacts with both A^a-3 and A^x (globo-A), but not (or very weakly) with A^a-1 and A^a-2, it is clear that it is reactive with the terminal A structure (Subsite 1 in Figure 7). Since it also reacts with the H_1-3 structure and weakly with Gal-A^a, the binding site must also encompass the internal A sequence (subsite 2 in Figure 7). The relatively strong binding to H_1-3 in comparison to Gal-A^a can be explained by the presence of the additional L-fucose residue in H_1-3, which contributes to the interaction through subsite 1. The results show that unlike previously described anti-A antibodies Ab M2 has a strong preference for binding type 3 and type 4 chains over type 1 and type 2 A chains.

Unexpectedly, Ab M2, even though it agglutinated all A erythrocytes, did not react with any of the A or AB salivas tested in the ELISA assay. In contrast, Abs HT29-36, CB, and CLH6 reacted with all A and AB salivas. That the nonreactivity of Ab M2 is due to a characteristic of the A determinants in saliva was shown by the fact that Ab M2 was able to react with human ovarian cyst fluid A glycoproteins. A probable explanation for these results is that A saliva mucins have type 1 and type 2 sequences (which are recognized by Abs CB, HT 29-36, and CLH6) but lack type 3 and type 4 sequences (which would be recognized by Ab M2).

These monoclonal antibodies (except for CB) were generated by immunization of mice with various human cancer cell lines. The fact that these antibodies react with different determinants belonging to the blood group A family suggests the possibility that cancer cells may express a variety of A antigenic determinants on their surfaces that cannot be distinguished by conventional reagents. With the availability of a panel of well-characterized anti-A blood group reagents it will

be possible to analyze the expression of A antigen variants in normal tissues and in tumor samples to search for alterations of expression in malignancy.

Added in Proof

Recently, Chen & Kabat (1985) and Gooi et al. (1985) have described the specificity of five and six mouse monoclonal anti-A antibodies, respectively. The results obtained by Chen and Kabat with the commercial anti-A antibody, the only moAb in common with this study, were essentially in agreement with our data. These two studies also indicate the ability of mouse moAbs to distinguish between the numerous A variant structures.

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