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A Monoclonal Antibody that Recognizes both Le^b and Y (Le^y) Antigens

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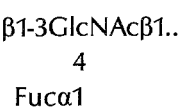
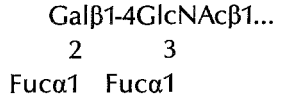
A hemagglutinating monoclonal antibody has been obtained from a mouse/mouse hybridoma after immunisation with the Le^b-active oligosaccharide, lacto-*N*-difucohexaose I, coupled to edestin. The antibody agglutinated human red cells regardless of Lewis phenotype. Blood group O cells were strongly agglutinated, and progressively weaker agglutination was observed with A_2, B and A_2B cells. Blood group A_1 and A_1B cells were not agglutinated.

By examining the binding of the antibody to glycolipids and oligosaccharides it was shown that the Le^b and Y (Le^y)-haptens bind to a similar extent. Full binding activity was dependent on the presence of both fucosyl residues.

Several monocloncal antibodies have been described that specifically bind the Le^b antigen [1-3]



or its isomer, the Y (also known as Le^y) antigen [4-9],



Abbreviations: LND I, lacto-*N*-difucohexaose I, $IV^2Fuc,III^4FucLcOse_4$; LND I-OL, lacto-*N*-difucohexaitol I.

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Table 1. Structures of oligosaccharides.

(1) lacto-*N*-difucohexaose I	Galβ1-3GlcNAcβ1-3Galβ1-4Glc 2 4 Fucα1 Fucα1
(2) lactodifucotetraose	Galβ1-4Glc 2 3 Fucα1 Fucα1
(3) 2'-fucosyllactose	Galβ1-4Glc 2 Fucα1
(4) 3-fucosyllactose	Galβ1-4Glc 3 Fucα1
(5) lacto-*N*-fucopentaose I	Galβ1-3GlcNAcβ1-3Galβ1-4Glc 2 Fucα1
(6) lacto-*N*-tetraose	Galβ1-3GlcNAcβ1-3Galβ1-4Glc
(7) lacto-*N*-fucopentaose II	Galβ1-3GlcNAcβ1-3Galβ1-4Glc 4 Fucα1

That antibodies can discriminate between Le^b and Y (Le^y) implies a difference in the shapes of the antigen molecules. Recent studies of the preferred three dimensional conformations of Le^b and Y (Le^y) conclude from nuclear magnetic resonance (NMR) data that these sugar chains differ substantially in some regions, in other regions they may share extensive topological similarities [10, 11]. These similarities have been invoked by Spohr *et al.* [10] to account for the Le^b-Y cross-reactivity observed for lectin IV of *Griffonia simplicifolia* and for some polyclonal anti-Le^b antisera. In the present report we describe a monoclonal antibody generated from splenocytes of a mouse immunized with a neoglycoconjugate containing the Le^b-active oligosaccharide, lacto-*N*-difucohexaose I (LND I) (see Table 1 for oligosaccharide structures), coupled to edestin. This antibody, designated 64/4D8, binds the Le^b and Y antigens about equally well and requires the presence of both fucosyl residues on each of the antigens for full binding activity.

Materials and Methods

Hybridoma 64/4D8 was obtained from a similar fusion experiment performed exactly as described for the anti-Le^b antibody-producing hybridoma 10.2, using the same batch of LNDI-edestin [12] and an identical immunization route [1]. Oligosaccharides were isolated from human milk as described by Kobata [13] and radiolabeled by reduction with tritiated sodium borohydride [14]. Glycolipids Le^a-5, Le^b-6, H-5-1 and A-7-1 were

Table 2. Structures of glycolipids.

H-5-1	Galβ1-3GlcNAcβ1-3Galβ1-4Glc-Cer 2 Fucα1
H-5-2	Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer 2 Fucα1
Le^a-5	Galβ1-3GlcNAcβ1-3Galβ1-4Glc-Cer 4 Fucα1
X-5	Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer 3 Fucα1
Le^b-6	Galβ1-3GlcNAcβ1-3Galβ1-4Glc-Cer 2 4 Fucα1 Fucα1
Y-6	Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer 2 3 Fucα1 Fucα1
A-7-1	GalNAcα1-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-Cer 2 4 Fucα1 Fucα1

isolated from human small intestine [15-18]; X-5 (Le^x), Y-6 (Le^y) and H-5-2 were isolated from dog small intestine [16, 19] (see Table 2 for glycolipid structures). Hemagglutination studies and ELISA were performed as described previously [1]. Radioimmunoassays to test oligosaccharides as inhibitors of binding of ^{3}H-LND I-OL using a nitrocellulose filter assay were performed as previously described [20] except that incubations were carried out for 16 h at 4°C.

Results

Antibody 64/4D8 gave positive hemagglutination reactions with all the O, A_2, and B red cells tested but negative or weak reactions with A_1, A_1B or A_2B cells, irrespective of Lewis blood group (Table 3). Agglutination reactions were enhanced by pre-treating the cells with papain. No significant enhancement of hemagglutination was observed when tests were performed at 4°C.

In ELISA tests, antibody 64/4D8 bound strongly to Le^b-6 and Y-6 glycolipid target antigens coated on microtiter plates. The antibody reacted only weakly with H-5-1 and H-5-2 glycolipids (see Table 2 for glycolipid structures) (Fig. 1), and not at all with X-5, Le^a-5 and

Table 3. Hemagglutination of erythrocytes from individuals of various ABO and Lewis blood groups by antibody 64/4D8.

Lewis blood group	ABO blood group					
	O	A_1	A_2	B	A_1B	A_2B
Le(a+b−)	+++[a]	−	++	+	−	(+)
Le(a−b+)	+++	−	++	+	−	(+)
Le(a−b−)	+++	−	++	+	−	(+)

[a] Strength of agglutination was estimated in a blind assay using coded target cells at a standard dilution of antibody giving a titer of 64 for O, Le(a−b−) cells.

A-7-1 glycolipids. Thus, antibody 64/4D8 specifically recognizes an epitope shared in common by the difucosylated oligosaccharides of glycolipids Le^b-6 and Y-6. Binding of 64/4D8 to Le^b-6 or Y-6 in microtiter wells is inhibited by LND I and lacto-difucotetraose (LDFT) at similar molar concentrations of the inhibitors (Fig. 2).

To determine relative binding affinities, various oligosaccharides were tested as inhibitors of ^{3}H-LND I-OL binding by 64/4D8 in a nitrocellulose filter radioimmunoassay (Fig. 3). The results agree with the relative activities of oligosaccharides as inhibitors of binding to glycolipids in ELISA in that LDFT and LND I are about equally effective as inhibitors of ^{3}H-LND I-OL binding. Other oligosaccharides containing only one fucosyl residue linked to either lactose or lacto-*N*-tetraose are less than 1% as active as the difucosylated analogues on a molar basis. The core oligosaccharides lacking fucose (lacto-*N*-tetraose, lacto-*N*-neotetraose and lactose) are inactive at the concentrations tested.

Discussion

The results of serological and immunochemical characterization indicate that monoclonal antibody 64/4D8 binds almost equally well to Le^b and Y (Le^y) antigens. Like other anti-Y monoclonal antibodies described previously [4-9], 64/4D8 strongly hemagglutinates type O human erythrocytes. Previous descriptions of the serological properties of anti-Y monoclonal antibodies have stated that the antibodies react only with human erythrocytes from individuals of blood group O, but it is not clear that cells from all ABO subgroups were tested. Antibody 64/4D8 reacts nearly as well with A_2 cells as it does with type O cells (Table 3). It reacts weakly with A_2B and B cells but fails to agglutinate A_1 and A_1B cells. The paradoxical observation that hemagglutination reactions are not influenced by Lewis blood group phenotype implies that the Y structure is the major target antigen that mediates hemagglutination by antibody 64/4D8. Although Le^b sites clearly are accessible for binding on cells that react positively with standard anti-Le^b typing sera, their density at the red cell surface, a critical factor for hemagglutination [21], may not be sufficient for the formation of stable agglutinates by 64/4D8.

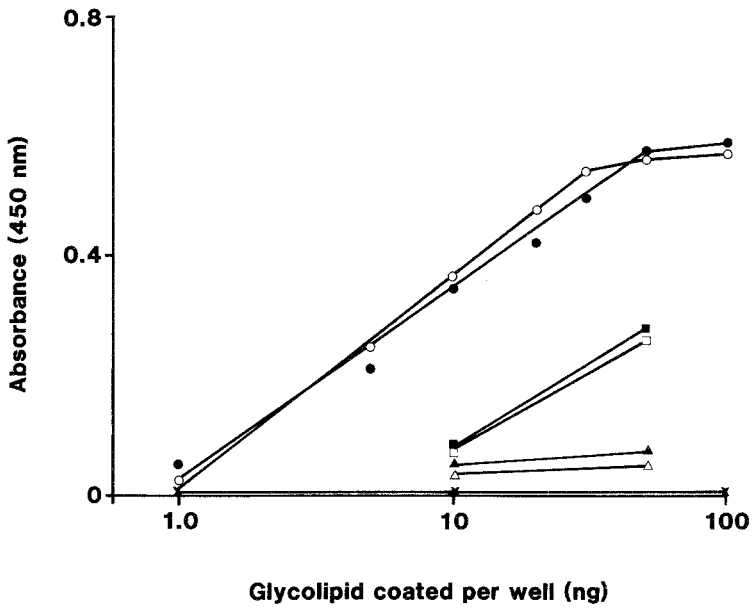

Figure 1. Binding of antibody in ascitic fluid from hybridoma 64/4D8 to microtiter plates coated with glycolipids Le^b-6 (●), Y-6 (○), H-5-1 (■), H-5-2 (□), Le^a-5 (▲), X-5 (△) or A-7-1 (x) measured using the ELISA technique.

The rather complex relationship between biosynthesis of blood group ALe^b and AY antigens and their specific recognition by monoclonal antibodies is not completely understood. It is well-established [22] that type 1 and type 2 precursor chains are not acceptors for the A enzyme until they are first converted to H-type 1 or H-type 2 structures by addition of a single fucosyl residue at the 2-O-position of terminal galactose. According to *in vitro* results using enzymes from diverse sources [22-24] the preferred route to biosynthesis of the difucosylated ALe^b or AY composite structures appears to be via addition of a second fucosyl residue to monofucosylated A-type 1 or A-type 2 structures. The alternative biosynthetic route has not been demonstrated; in fact, both type 1- and type 2-derived difucosylated oligosaccharides with Le^b or Y activities are reported to be poor acceptors for the A enzyme [25, 26].

On the basis of the above evidence for the pathways of biosynthesis for ALe^b and Y antigens (which unfortunately do not include coordinately regulated enzymes in a single organism or cell type) it seems likely that varied expression of the Y determinant on cells from donors of different ABO blood groups is the end result of competition for the H-type 2 precursor between the A enzyme and GDP-fucose:*N*-acetylglucosamine α(1-3)-fucosyltransferase. Monoclonal antibodies with anti-A Le^b and anti-AY specificities have also been reported [27]. We are not aware of any systematic study comparing reactivities of various anti-A monoclonal antibodies with the AY composite antigen. It has been shown for conventional anti-A sera in quantitative precipitin inhibition assays using oligosaccharides derived from soluble blood group substances that the difucosylated A-type 2 chain (AY) is a much less active inhibitor than the monofucosylated A type 2 chain [28].

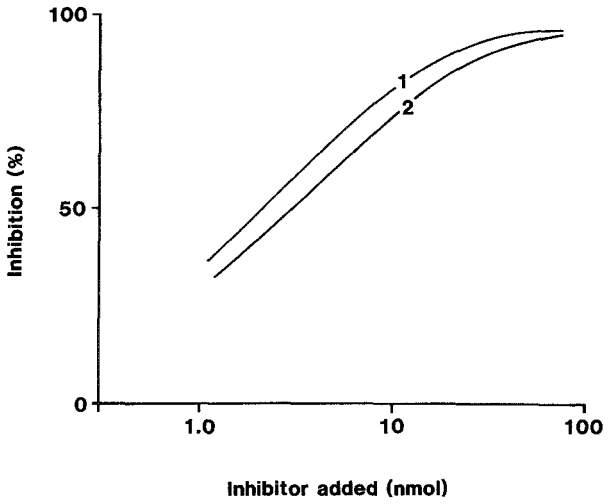

Figure 2. Inhibition of binding of antibody in culture supernatant from hybridoma 64/4D8 to glycolipid Le^b-6 by oligosaccharides lacto-*N*-difucohexaose I (1) and lactodifucotetraose (2).

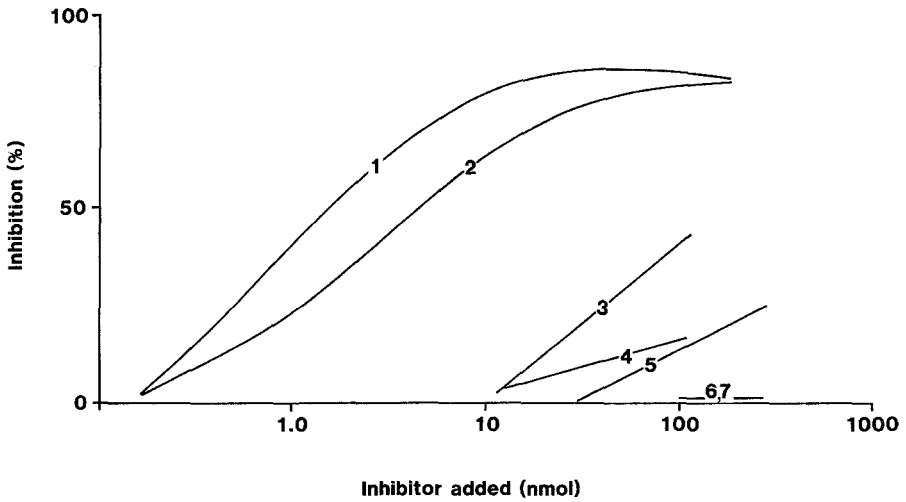

Figure 3. Inhibition of binding of ^{3}H-lacto-*N*-difucohexaitol I to hybridoma antibody 64/4D8 by oligosaccharide: See Table 1 for oligosaccharide structures.

Several monoclonal antibodies specific for the Y antigen have been described [4-9]. When used in radioimmune overlay on thin-layer plates, one of these antibodies detected the Y-6 glycolipid as well as a series of higher structures present in the total neutral glycolipids of human O red cells, but failed to detect any of these Y-active components in a similar glycolipid fraction from A erythrocytes (6). We have not had purified AY glycolipid antigen available to test as a target antigen for antibody 64/4D8 but the observations that the antibody fails to bind ALe^b-7 and that Le^b and Y strongly crossreact (Fig. 1) suggest that recognition of AY by this antibody is blocked by terminal α(1-3)-linked *N*-acetylgalactosamine.

Computer modeling of the three dimensional structures of complex carbohydrates using NMR measurements and HSEA calculations [29] has detected congruent topological features shared by the Le^b and Y antigens, which lead to the prediction that Le^b and Y could strongly cross react [10, 30]. It seems reasonable to interpret the cross reaction of antibody 64/4D8 with these two antigens as recognition of an epitope that includes these topological similarities. Cross-reactive recognition of Le^b and Y antigens by type IV *Griffonia simplicifolia* lectin [10] and partial cross-reactivity by some, but not all, anti-Le^b antibodies has been explained by Lemieux and coworkers on the same basis [31]. Relatively high frequency of cross-reacting anti-Le^b/anti-Y antibodies in human and animal anti-Le^b sera have also been reported [32].

Antibody 64/4D8 has been studied as an immunohistochemical reagent to detect antigenic changes associated with malignant and pre-malignant proliferative lesions of the colonic mucosa [33]. Results showing the presence of detectable target antigen in 30 of 30 colonic carcinomas and its absence from normal colonic mucosa agree with the results of similar studies [34, 35] using other anti-Y monoclonal antibodies. Further study of the structural and metabolic basis for regulation of Le^b and Y expression in human tissues may provide useful insights into chemical changes associated with malignant transformation.

References

1 Messeter L, Brodin T, Chester MA, Karlsson K-A, Zopf D, Lundblad A (1984) Vox Sang 46:66-74.
2 Blaszczyk M, Hansson GC, Karlsson K-A, Larson G, Strömberg N, Thurin J, Herlyn M, Steplewski Z, Koprowski H (1984) Arch Biochem Biophys 233:161-68.
3 Brockhaus M, Magnani JL, Herlyn M, Blaszczyk M, Stelpewski Z, Koprowski H, Karlsson K-A, Larsson G, Ginsburg V (1981) J Biol Chem 256:13223-25.
4 Ginsburg V, Magnani JL, Spitalnik SL, Spitalnik PF, Roberts DD, Dubois C (1985) Proc 8th Int Symp Glycoconjugates, eds Davidson EA, Williams JC, Di Ferrante NM, Praeger, New York, p 550-51.
5 Blaineau C, Le Pendu J, Arnaud D, Connan F, Avner P (1983) EMBO J 2:2217-22.
6 Abe K, McKibbin JM, Hakomori S (1983) J Biol Chem 258:11793-97.
7 Brown A, Feizi T, Gooi HC, Embleton MJ, Picard JK, Baldwin RW (1983) Biosci Rep 3:163-70.
8 Lloyd KO, Larson G, Strömberg N, Thurin J, Karlsson K-A (1983) Immunogenetics 17: 537-41.
9 Le Pendu J, Fredman P, Richter ND, Magnani JL, Willingham MC, Pastan I, Oriol R, Ginsburg V (1985) Carbohydr Res 141:347-49.

10 Spohr U, Hindsgaul O, Lemieux RU (1985) Can J Chem 63:2644-52.
11 Spohr U, Morishima N, Hindsgaul O, Lemieux RU (1985) Can J Chem 63:2659-63.
12 Zopf DA, Tsai CM, Ginsburg V (1978) Methods Enzymol 50:163-69.
13 Kobata A (1972) Methods Enzymol 28:262-71.
14 Kobata A, Ginsburg V (1972) Arch Biochem Biophys 150:273-81.
15 Smith EL, McKibbin JM, Karlsson K-A, Pascher I, Samuelsson BE, Li Y-T, Li S-C (1975) J Biol Chem 250:6059-64.
16 McKibbin JM, Spencer WA, Smith EL, Månsson J-E, Karlsson K-A, Samuelsson BE, Li Y-T, Li S-C (1982) J Biol Chem 257:755-60.
17 Breimer ME, Karlsson K-A, Larson G, McKibbin JM (1983) Chem Phys Lipids 33:135-44.
18 Falk K-E, Karlsson K-A, Samuelsson BE (1979) Arch Biochem Biophys 192:191-202.
19 Smith EL, McKibbin JM, Karlsson K-A, Pascher I, Samuelsson BE, Li S-C (1975) Biochemistry 14:3370-76.
20 Zopf DA, Ginsburg A, Ginsburg V (1975) J Immunol 115:1525-29.
21 Tsai C-M, Zopf D, Ginsburg V (1978) Biochem Biophys Res Commun 80:905-10.
22 Kobata A, Ginsburg V (1970) J Biol Chem 245:1484-90.
23 Samuelsson B (1983) FEBS Lett 152:305-10.
24 Schwyzer M, Hill RL (1977) J Biol Chem 252:2346-55.
25 Kobata A, Grollman EF, Ginsburg V (1968) Arch Biochem Biophys 124:609-12.
26 Hearn WM, Smith ZG, Watkins WM (1968) Biochem J 109:315-17.
27 Clausen A, McKibbin JM, Hakomori S, (1985) Biochemistry 24:6190-94.
28 Lundblad A, Kabat EA (1971) J Immunol 106:1572-77.
29 Lemieux RU, Bock K, Delbaere LTJ, Koto S, Rao VS (1979) Can J Chem 58:631-53.
30 Thogersen H, Lemieux RU, Bock K, Meyer B (1982) Can J Chem 60:44-57.
31 Lemieux RU, Le Pendu J and Norberg T (1980) in Joint Congr Int Soc Hematol and Int Soc Blood Transf, Montreal, Canada, p 261.
32 Francois A, Sansonetti N, Mollicone R, LePendu J, Galton J, Jaulmes B, Oriol R (1986) Vox Sang 50:227-34.
33 Enblad P, Glimelius B, Busch C, Påhlman L, Pontén J, Chester MA, Lundblad A (1986) Anticancer Res 6:139-46.
34 Brown A, Ellis IO, Embleton MJ, Baldwin RW, Turner DR, Hardcastle JD (1984) Int J Cancer 33:727-36.
35 Kim YS, Yuan M, Itzkowitz SH, Sun Q, Kaizy T, Palekar A, Trump BF, Hakomori S (1986) Cancer Res 46:5985-92.