Volume 307, number 3, 283–286 FEBS 11357 August 1992

Oligosaccharide-binding molecules on the surface of human hemopoietic and lymphoid cells

I.V. Abramenko[a], D.F. Gluzman[a], E.Yu. Korchagina[b], T.V. Zemlyanukhina[b] and N.V. Bovin[b]

[a]*Kavetsky Institute for Oncology and Radiobiology Problems, Ukraine Academy of Sciences, Kiev-22, 252022 Kiev, Ukraine* and [b]*Shemyakin Institute of Bioorganic Chemistry, Russian Academy of Science, Moscow-437, 117871 Moscow, Russia*

Received 2 June 1992

High oligosaccharide specificity for binding carbohydrate probes (biotinylated polyacrylamide with carbohydrates attached) with human hemopoietic and lymphoid cells is shown. Of 15 probes studied those bearing blood group trisaccharides, A and B, bound most intensely. In addition, transformed (leukemic and lymphoid) cells interacted more strongly than normal ones.

Lectin; Endogenous; Oligosaccharide probe; Malignancy; Leukocyte–endothelial cell adhesion molecule; Blood group

1. INTRODUCTION

Participation of lymphoid and other hemopoietic cells in normal and pathological processes, such as recirculation, homing, neutrophil–platelet and leucocyte–endothelium adhesion, cluster formation, inflammation, leucocyte adhesion disorder, etc. are mediated in many respects by the interaction of cell–surface lectin-like molecules [1] decoding the information found in glycoconjugates on the surface of other cells [2–4]. Carbohydrate–lectin interactions play an essential part in the development of hemopoietic malignancies and the spreading of malignant cells which express specific lectins [5,6].

Carbohydrates of cell surface glycoconjugates are usually oligosaccharides. Thus there is the need for molecules which decode the structural information of complex carbohydrates to have oligo- rather than monosaccharide specificity.

The latest studies confirm the presence of functionally significant lectin-like molecules on the surface of many cells, in particular a family of leukocyte–endothelium cell adhesion molecules (LECAMs or selectins) which recognize SiaLe^x and related oligosaccharides [2–4,7], as well as receptors of lysogangliosides, GM1 and GT1b [8,9]. Nevertheless, the specificity of cell surface lectins is usually characterized with the help of monosaccharides or monosaccharide-based probes. In this study the presence of oligosaccharide-binding molecules on the surface of normal and malignant hemopoietic and lymphoid cells varying in differentiation levels is described.

Abbreviations: PAA, polyacrylamide; DMF, dimethylformamide; PBS, phosphate buffered saline; LECAM, leukocyte–endothelial cell adhesion molecules.

Correspondence address: N.V. Bovin, Shemyakin Institute of Bioorganic Chemistry, Russian Academy of Science, Moscow-437, 117871 Moscow, Russia.

2. MATERIALS AND METHODS

Peripheral blood mononuclears from 10 normal individuals and 27 patients having different hemopoietic malignancies (see Table III). The presence of transformed cells (the study was made before therapy began) was not less than 90%. Mononuclears were isolated by gradient centrifugation with Ficoll-verographin (d = 1.076–1.078). Cells were washed thrice with Hank's balanced salt medium. Experiments on binding inhibition of the TF probe were carried out on lymphoid tonsil cells, as well as the mice lymphoid cell lines, P-338 and EL-4. All low molecular weight inhibitors were added to solutions of probes in a tenfold concentration compared to the probe. Cytological preparations of approximately 4×10^6 cells were dried, fixed in 10% formalin vapor for 3 min and incubated with probe solution (0.1–1 mg/ml in PBS) for 1 h in a moist chamber at room temperature. After three washes with PBS, incubation with streptavidin–peroxidase or streptavidin–alkaline phosphatase solution (Amersham, 1:1000) was performed under the same conditions. Visualization of the bound probes was achieved by 3,3′-diaminobenzidine/H_2O_2 reaction or naphtol AS-TR phosphate (Fast red TR) [10].

2.1. *Probes*

A solution containing 11 μmol of saccharide (as 3-aminopropyl glycoside, for synthesis see [11,12] in 0.6 ml of DMF was added to 11 mg (55 μg eq.) of poly(4-nitrophenylacrylate) [13] in 0.4 ml of DMF followed by addition of 20 μl of triethylamine and 1.3 mg (2.8 μmol) of biotin–$NH(CH_2)_6NH_2$ trifluoroacetate (Bioprocess, Moscow) in 0.1 ml of DMF. The mixture was incubated for 24 h at 40°C and 50 μl of ethanolamide was added. After a further 24 h the mixture was applied to the column with Sephadex LH-20, and the probe was eluted with acetonitrile/water in a 1:1 ratio; preparative yields 90–95%, yields of coupling 100%. The M_r's defined on a TSK HW-53J column using proteins as standards were 26–32 kDa.

Table I

Designation and structure of probes

Designation	Structure	Designation	Structure
TF	Galβ1-3GalNAcα	GalNAcα	GalNAcα
H	Fucα1-2Galβ1-3GlcNAc	F3GN	Fucα1-3GlcNAc
F4GN	Fucα1-4GlcNAc	Fucα	Fucα
B	Galα1-3 Gal Fucα1-2	Le^a	Fucα1-4 GlcNAc Galβ1-3
B_{di}	Galα1-3Gal	LacN	Galβ1-4GlcNAc
Galβ	Galβ	Lac	Galβ1-4Glc
A	GalNAcα1-3 Gal Fucα1-2	A_{di}	GalNAcα1-3Gal

3. RESULTS AND DISCUSSION

Since the affinity of target oligosaccharide-binding molecules was believed to be low, the probes (Table I) were designed to give maximum binding: (i) a high saccharide density on the polymer was created (20 mol%); (ii) PAA was taken to be a matrix composed of a random coil capable of re-arranging itself for the recognizing molecule; (iii) the biotin label was included to provide effective probe development. Lack of interaction of all the cells studied with the β-Glc probe shows that non-specific sorption was low. At the same time high oligosaccharide specificity was found and is demonstrated here by the disaccharide probe, TF (carbohydrate ligand, Galβ1-3GalNAcα). It may be seen from Table II that TF binding is inhibited only by α-disaccharide and not by β-disaccharide or monosaccharides (Galβ-OCH_3, GalNAcα-sp).

For heterogeneous cell populations, as in the given case, the correlation of probe couples binding (χ^2) may also be the specificity criterion. The χ^2 value was calculated for all the probe couples based on data of probes binding with the cells from all 40 donors (see Table III plus 10 healthy donors). The only statistically reliable χ^2 values for the probes given in Table I are the following:

χ^2	χ^2
TF→4.4←Fucα	Le^a→5.8←Galβ
H→4.5←Lac	F4GN→9.3←LacN
Le^a→4.5←Lac	B→4.6←Galβ
Le^a→6.9←F4GN	B_{di}→4.2←A_{di}

It can be seen that there is a lack of correlation between both TF↔Galβ and TF↔GalNAcα probe couples. The only probe which reliably correlates with TF is Fuc. These may be two explanations of this rather unexpected fact: (i) cell lectin recognizes the structure Fucα1-2Galβ1-3GalNAcα (H type 3); (ii) for some as yet unknown reason the expression of TF- and Fuc-specific sites on the cell surface is concordant. These possibilities could be checked by synthesizing probe H (type 3).

A group of probes binding correlately with cells, including Le^a, F4GN, Galβ and LacN, attracts the attention. The structures mentioned are the fragments of oligosaccharides, $SiaLe^x$ and $SiaLe^a$, which are LECAM receptors. The latter bind not only with complete receptors, $SiaLe^x$/$SiaLe^a$, but also (to a lesser extent) with their fragments and some related receptors, Le^x, Le^a, Le^y [4]. It is possible that in this study we also found a lectin(s) from the same family.

Binding of probes A and B, where the active ligands are determinant blood group trisaccharides A and B, is of special interest. Antigens A and B are the main erythrocyte antigens but they also appear on endothelial cells ('histo' blood groups A and B) [14]. A soluble rat lung lectin has been described that interacts with oligosaccharide A [15]. Probably the finding of antigens A and B cell receptors will help to understand their function. It is possible that histo-antigens A and B and proteins binding them are partners in intercellular recognition analogous to that of LECAMs–$SiaLe^x$. High specificity of A and B probe binding ensures from that (i) trisaccharide A inhibits probe A binding, trisaccharide B inhibits binding of probe B and not vice versa (inhibition was performed as in the case of the TF probe), (ii) there is no correlation between binding of probes A and B

Table II

Inhibition of binding cell lines P-338 and EL-4 and also cells from tonsils with TF probe (0.1 mg/ml) by low molecular weight sugars (0.15 nM)

Inhibitor	Degree of inhibition
Galβ1-3GalNAcα1-sp	+
Galβ1-3GalNAcα1-$OCH_2C_6H_5$	+
Galβ1-3GalNAc	±
Galβ1-OCH_3	±
Galβ1-3GalNAcβ-sp	−
GalNAcα1-sp	−

+, complete inhibition for all cells; ±, partial inhibition; −, no inhibition.
sp = $OCH_2CH_2CH_2NHCOCF_3$

Table III

Probe interaction with hemoblastose patient cells and histiocytes from exudate of non-malignant patients

n	Disease or cells*	Probes													
		TF	H	Fucα	Le^a	Galβ	B	LacN	Lac	A_{di}	F3GN	GalNAcα	F4GN	B_{di}	A
1	pre-T-ALL	+	−	−	+	+	+	−	−	−	−	−	+	−	−
2	pre-T-ALL	+	+	+	−	+	+	−	+	+	+	−	−	+	+
3	C-ALL	−	+	−	−	−	−	−	+	−	+	nd	−	−	+
4	C-ALL	+	−	+	−	−	+	−	+	−	−	−	−	−	+
5	C-ALL	+	−	−	−	−	+	−	−	−	+	−	−	−	+
6	C-ALL	+	+	+	+	+	+	−	+	+	+	+	+	−	+
7	C-ALL	+	−	−	−	+	+	−	+	+	+	−	−	−	+
8	C-ALL	+	+	+	+	+	−	−	+	+	+	−	−	−	−
9	CLL	−	−	−	−	+	+	−	−	+	−	nd	−	−	+
10	CLL	−	−	−	−	−	+	−	−	+	+	−	−	+	+
11	CLL	−	−	−	−	−	−	−	−	+	−	+	−	+	+
12	HCL	−	−	−	−	+	+	−	−	nd	−	−	−	+	+
13	HCL	+	−	−	−	+	+	−	−	+	+	−	−	+	−
14	NHL	+	−	+	+	+	−	−	+	+	+	−	−	+	+
15	NHL	−	−	−	+	+	+	+	+	+	+	−	+	+	+
16	NHL	−	−	−	+	+	+	+	+	−	+	+	−	−	+
17	NHL	−	+	+	+	+	+	+	−	−	+	nd	+	+	+
18	NHL	−	+	−	+	+	+	+	+	+	−	−	+	−	+
19	PL	+	+	−	+	+	+	+	+	−	+	nd	+	−	+
20	AML	−	−	+	+	+	−	−	−	−	−	−	−	−	−
21	AML	−	−	−	+	−	−	−	+	+	−	−	−	+	−
22	AML	−	−	+	+	−	−	−	−	−	−	−	−	−	−
23	AMML	+	−	+	−	+	−	−	−	+	+	−	−	+	−
24	AMML	+	−	+	−	−	−	−	+	+	+	+	+	+	−
25	AMML	+	+	−	−	−	+	−	+	+	+	−	−	+	−
26	AMonL	+	+	+	+	+	+	−	+	−	+	−	−	+	+
27	AMonL	+	+	+	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd
28	Histiocyte	+	−	+	−	+	+	−	−	+	−	−	−	−	+
29	Histiocyte	+	+	+	−	+	+	−	−	+	+	−	−	+	+
30	Histiocyte	+	+	+	−	+	+	−	−	+	+	−	−	+	+

+, probe reacts with more than 90% of cells; −, no reaction; nd, not determined.

*Acute lymphoblastic leukemia (ALL), common acute lymphoblastic leukemia, plasmoblastic leukemia (PL), acute myeloblastic leukemia (AML), acute myelomonoblastic leukemia (AMML), acute monoblastic leukemia (AMonL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), B-cell forms of non-Hodgkin's lymphomas in leukemization stage (NHL).

with each other and also with A_{di}, B_{di}, H. It is interesting that transformed cells bind probes A and B more intensely than normal ones, while there is no correlation between binding and relationship of donor to the A/B group.

During this work an analysis of correlation between cell phenotype and expression of oligosaccharide-binding molecules on their surface was carried out (Table III). Briefly the results obtained may be summarized as follows: (i) the expression of carbohydrate-binding molecules on different types of hemopoietic and lymphoid cells varies depending on cell type, their differentiation level and the mitotic cycle phase; (ii) expression variability is high, and only some of the probes are cell- or disease specific (e.g. F4GN is preferable for the cells from patients having PL and NHL); (iii) A and B of the probes used interacted most intensely and with more types of cells (B-lymphocytes, T-lymphocytes, mononuclear phagocytes).

Thus, specific complex carbohydrate receptors are exposed on the surface of hemopoietic and lymphoid cells, moreover, the degree of expression on malignant cells is considerably higher.

REFERENCES

[1] Kieda, M.T., Bowles, D.J., Ravid, A. and Sharon, N. (1978) FEBS Lett. 94, 391–396.
[2] Stoolman, L.M. (1989) Cell 56, 907–910.
[3] Brandley, B.K., Swiedler, S.J. and Robbins, P.W. (1990) Cell 63, 861–863.
[4] Feizi, T. (1991) Trends Biochem. Sci. 16, 84–86.
[5] Gabius, H.-J. (1988) Angew. Chem. Int. Ed. Engl. 27, 1267–1276.
[6] Gabius, H.-J. (1987) Cancer Invest. 5, 39–46.
[7] Waltz, G., Aruffo, A., Kolanus, W., Bevilacqua, M. and Seed, B. (1990) Science 250, 1132–1135.
[8] Gabius, S., Kayser, K., Hellman, K.P., Ciesiolka, T., Trittin, A. and Gabius, H.-J. (1990) Biochem. Biophys. Res. Commun. 169, 239–244.

[9] Tiemeyer, M., Yoshinobu, Y. and Schnaar, R.L. (1989) J. Biol. Chem. 264, 1671–1681.
[10] Yam, L.T., Jankila, A.J. and Li, C.-Y. in: Advances in Immunohistochemistry (R.A. Dellelis ed.) p. 352, 1988, Raven Press.
[11] Bovin, N.V., Zemlyanukhina, T.V. and Khorlin, A. Ya. (1985) Bioorgan. Khim. 11, 1256–1264.
[12] Korchagina, E. Yu. and Bovin, N.V. (1991) Bioorgan. Khim. 18, 283–298.
[13] Matrosovich, M.N., Mochalova, L.V., Marinina, V.P., Byramova, N.E. and Bovin, N.V. (1990) FEBS Lett. 272, 209–212.
[14] Clausen, H. and Hacomori, S. (1989) Vox. Sang. 56, 1–20.
[15] Leffler, H. and Barondes, S.H. (1986) J. Biol. Chem. 261, 10119–10126.