SPECIFIC INTERACTION OF PEANUT AGGLUTININ WITH THE GLYCOLIPID ASIALO GM1

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1. Introduction

Membrane glycoproteins that are involved in the antigenicity of cell surface have been used as specific markers for characterizing various subpopulations of lymphocytes [1]. Their glycoconjugate portions have different affinities for various lectins. Therefore, lectins with different sugar specificities are capable of characterizing the glycoproteins on cell surface membranes [2].

Abbreviations: PBS, phosphate-buffered saline; buffer A, PBS containing 0.1% (w/v) sodium deoxycholate; buffer B, PBS containing 0.05% (w/v) Tween 20; buffer C, buffer B containing 2% (w/v) BSA; BSA, bovine serum albumin; PNA, peanut agglutinin; TLMA, rat T lymphocyte macrophage-associated antigen; ELISA, enzyme-linked immunosorbent assay; NMR, nuclear magnetic resonance

Glycolipid abbreviations: GlcCer, Glc(β1–1)Cer; LacCer, Gal(β1–4)Glc(β1–1)Cer; CTH, $GbOse_3Cer$ = Gal(α1–4)Gal-(β1–4)Glc(β1–1)Cer; globoside, $GbOse_4Cer$ = GalNAc-(β1–3)Gal(α1–4)Gal(β1–4)Glc(β1–1)Cer; Forssman hapten glycolipid, IV^3-α-GalNAc-$GbOse_4Cer$ = GalNAc(α1–3)-GalNAc(β1–3)Gal(α1–4)Gal(β1–4)Gal(β1–1)Cer; asialo-GM1, $GgOse_4Cer$ = Gal(β1–3)GalNAc(β1–4)Gal(β1–4)Glc-(β1–1)Cer; GM1, II^3-α-NeuAc-$GgOse_4Cer$ = Gal(β1–3)-GalNAc(β1–4)Gal(β1–4)Glc(β1–1)Cer;

(3–2α)NeuAc

GD1a, II^3, IV^3-α-NeuAc-$GgOse_4Cer$ = Gal(β1–3)GalNac-

(3–2α)NeuAc

(β1–4)Gal(β1–4)Glc(β1–1)Cer;

(3–2α)NeuAc

fucosyl GM1, II^3-α-NeuAc, IV^3-α-Fuc-$GgOse_4Cer$ = Gal(β1–3)GalNAc(β1–4)Gal(β1–4)Glc(β1–1)Cer; asialo

(2–1α)Fuc (3–2α)NeuAc

GM2, $GgOse_3Cer$ = GalNAc(β1–4)Gal(β1–4)Glc(β1–1)Cer

Lectins have the ability to bind with glycolipids as well as with glycoproteins [3,4]. Peanut agglutinin (PNA) which has a specificity for Gal(β1–3)GalNac [5] has been used to characterize thymocyte subpopulations [6]. Although the Gal(β1–3)GalNAc structure is present in many soluble glycoproteins among membrane glycocompounds, it has been identified in a few glycoproteins such as glycophorin, and in glycosphingolipids, GM1 and asialo GM1.

On the other hand, antibody against asialo GM1, whose oligosaccharide structure is Gal(β1–3)GalNAc-(β1–4)Gal(β1–4)Glc, has been used as a probe capable of distinguishing immunologically competent cells of a particular type, such as natural killer cells, fetal thymocytes and suppressor cells of mice [7–10]. The antibody also reacts with the mouse leukemia-derived, macrophage-like cells, M-1 [11], mouse lung macrophages [12], mouse activated macrophages [13], some of rat macrophages and T cells [14,15]. Therefore, it is of interest to compare the reactivity of PNA to glycolipids with the reaction of anti-asialo GM1 antibody to glycophorin, and to investigate the correlation between anti-asialo GM1 positive and PNA-receptor positive cells.

2. Materials and methods

Glycophorin from human erythrocytes was generously donated by Dr M. Tomita (Showa University, Tokyo). GlcCer, LacCer, CTH and globoside were prepared from human erythrocyte membranes [16]. Forssman hapten glycolipid was from sheep erythrocytes [17]. Asialo GM1 was obtained by partial hydrolysis of GM1 with 1 N formic acid (100°C for 1 h) [18]. GM1 and GD1a were from bovine brain [19],

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and fucosyl GM1 was from boar testis [20]. Highly specific anti-asialo GM1 IgG antibody was prepared by affinity column chromatography on asialo GM1 oligosaccharide Sepharose 4B as in [21]. This purified antibody showed no cross-reaction with other glycolipids such as globoside, GM1, asialo GM2, fucosyl GM1 and GD1a [22].

2.1. *Enzyme-linked immunosorbent assay (ELISA)*

Reactivities of PNA (E. Y. Laboratory, San Mateo CA) and anti-asialo GM1 antibody with glycolipids and glycophorin were examined by the ELISA method essentially as in [23] with the modification of using buffer A for coating the micro ELISA-plate (Dynatech AG, Kloten) with the glycolipids [24]. Briefly, 50 μl antigen solution (10 μg glycolipids in 1 ml buffer A) was added to microplate (96 wells) and incubated at 37°C for 1 h for antigen adsorption. Then the plate was washed 3 times with buffer B. Buffer A (50 μl) solution containing PNA at different concentrations was added to the plate, which was incubated at 37°C for 30 min. After another washing with buffer B, antibody against PNA (200 dilution, E. Y. Laboratory) was added, incubation was continued for another 30 min at 37°C, and the plate was washed with buffer B. Peroxidase-conjugated goat anti-rabbit IgG (Miles Lab. Slough) in buffer C (50 μl) was added and incubation continued for 30 more min at 37°C; 300 μl 3% (w/v) 5-amino salicylic acid in 0.05% H_2O_2 solution was added as a substrate and after 1 h, the absorption at 450 nm and at 660 nm was read.

In the case of glycophorin, 50 μl untreated or neuraminidase-treated glycophorin in PBS (10 μg/ml or 1 μg/ml) was used for the adsorption to the microplate. The reaction with anti-asialo GM1 was examined in the same way as above. Glycophorin (500 μg) was incubated with 100 μU of neuraminidase (Boehring, Mannheim from *V. cholerae*) in 50 mM acetate buffer (pH 5.1) at 37°C for 24 h.

2.2. *Direct binding of PNA to glycolipids on thin-layer chromatogram*

The method originally developed in [25] was modified for application to a precoated silica gel thin-layer 60 glass plate (E. Merck AG, Darmstadt) as detailed in [26]. Briefly, ~1 μg glycolipid was applied on a thin-layer plate as a band <5 mm wide in each lane (1 × 9 cm). After development with chloroform/methanol/water = 60:35:8 (by vol.), the plate was air-dried completely, and PBS containing 1% (w/v) polyvinyl pyrolidone was carefully layered on the plate. The remaining solution on the plate was removed by aspiration. This procedure was repeated twice more, and 1 ml PBS containing 50 μg ^{125}I-labelled PNA (100 000 dpm) which was prepared by using Na^{125}I (14.1 Ci/mmol, Amersham) and chloramine T [27], was carefully overlayered and allowed to stand for 24 h at 4°C. The plate was dipped into 500 ml acetone to remove water. The air-dried plate was then exposed to Kodak X-O mat film at −80°C for 2 h.

2.3. *Cytotoxicity test for rat thymocytes*

PNA receptor-positive and PNA receptor-negative cells were prepared as in [6]. Cytotoxicity of anti-asialo GM1 antibody for rat thymocytes and PNA receptor-positive and PNA receptor-negative cells was tested by a method using a Coulter counter technique [28].

2.4. *PNA–Sepharose affinity column chromatography*

[^{3}H]GM1 and [^{3}H]asialo GM1 (10 mCi/mmol) which was prepared by using KB^3H_4 (113 mCi/mmol) and $PdCl_2$ as the catalyst [29,30], were dissolved in 200 μl buffer A. Ten microliters each of these glycolipid (~100 000 dpm) solutions was applied on a PNA–Sepharose column (1 ml bed vol., E. Y. Laboratory) which was equilibrated with buffer A. After application, the PNA–Sepharose column stood for 1 h at room temperature for adsorption. Buffer A (2 ml) was applied for washing and the retained material was eluted with 4 ml 0.5 M galactose-containing buffer A. The eluates were collected in test tubes in 200 μl.

3. Results and discussions

Reactivities of PNA and anti-asialo GM1 antibody with glycolipids and glycoproteins, respectively, were examined by ELISA. PNA interacted only with asialo GM1, but not with other glycolipids (fig.1a). Anti-asialo GM1 antibody also preferentially recognized neuraminidase-treated glycophorin (fig.1b).

Glycophorin which is the major erythrocytes membrane glycoprotein, is known to have O-serine- or -threonine-linked Gal(β1–3)GalNAc residues [31]. However, the oligosaccharides are usually sialylated at the 3rd position of the terminal galactose or 6th position of *N*-acetyl galactosamine. Therefore, the above result could be easily explained by postulating that

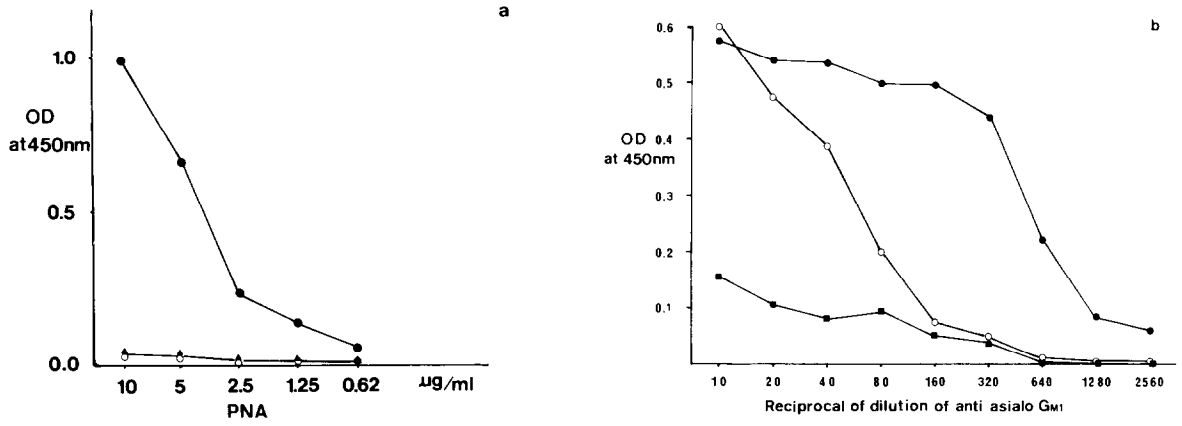

Fig.1. Interactions of PNA and anti-asialo GM1 antibody with glycolipids and glycophorin investigated by enzyme-linked immunosorbent assay. (a) Interaction of PNA with glycolipids: (●——●) asialo GM1; (○——○) GM1; (▲——▲) asialo GM2, fucosyl GM1, Forssman hapten glycolipid, globoside, and GD1a. (b) Interaction of anti-asiolo GM1 with glycophorin and asialo GM1: (●——●) asialo GM1; (■——■) glycophorin; (○——○) neuraminidase-treated glycophorin.

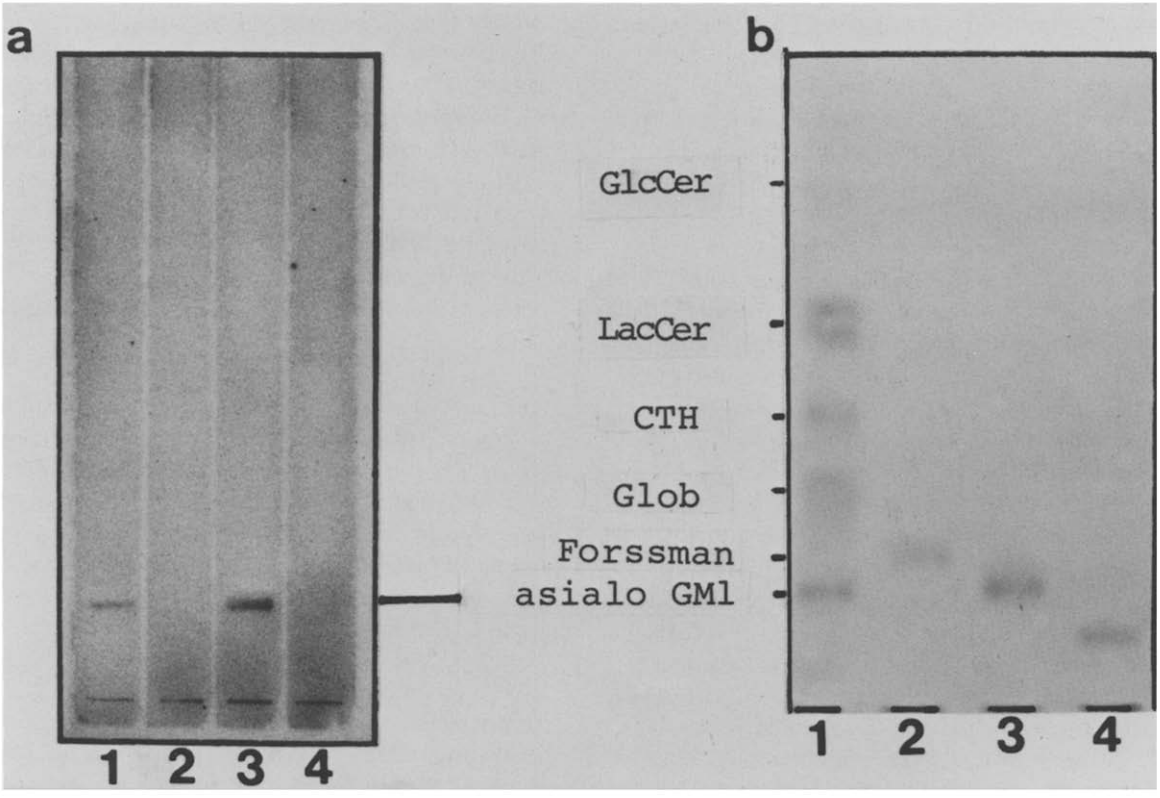

Fig.2. Direct visualization of the interaction of asialo GM1 by ^{125}I-labelled PNA on thin-layer chromatogram. (a) Autoradiography: (1) mixture of GlcCer, LacCer, CTH and Globoside; (2) Forssman hapten glycolipid; (3) asialo GM1; (4) GM1. (b) Thin-layer chromatogram identical with that in (a), but visualized with orcinol reagents (115°C, for 15 min).

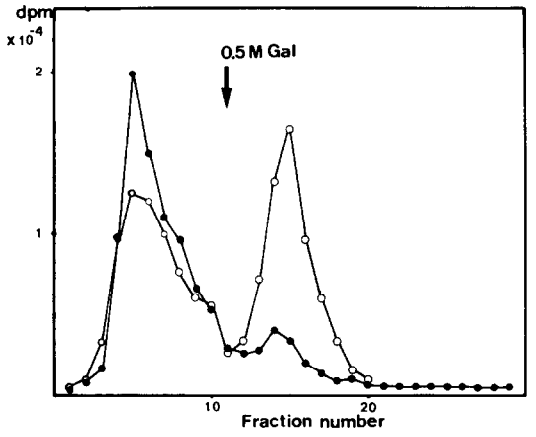

Fig.3. PNA-conjugated Sepharose affinity chromatography: (→) addition of 0.5 M galactose solution to the column; (○——○) asialo GM1; (●——●) GM1.

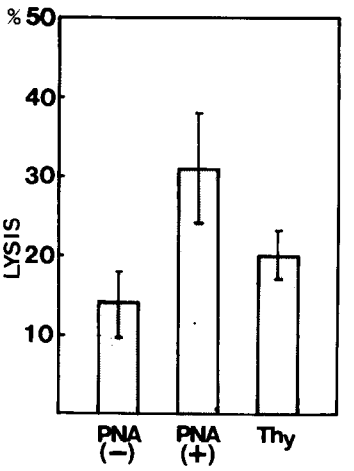

Fig.4. Cytotoxicity test on rat thymocytes and PNA receptor-positive and PNA receptor-negative cells with anti-asialo GM1 antibody and complement. The assay procedure is described in the text.

neuraminidase treatment caused the galactose residue to become susceptible to binding with anti-asialo GM1 antibody, and this result may explain why TLMA antigen, later identified as asialo GM1, which was not detected in rat erythrocytes, appeared after neuraminidase treatment at the cell surface [14].

Direct binding of PNA with asialo GM1 was shown on thin-layer chromatogram (fig.2). LacCer, CTH, globoside and Forssman hapten glycolipids, the terminal sugar of which is either galactose or *N*-acetylgalactosamine, did not interact with PNA. Furthermore, no interaction of PNA with GM1 was observed, although cholera toxin could specifically interact with GM1 under the conditions used.

The difference in specificity of PNA towards asialo GM1 and GM1 was further confirmed by PNA–Sepharose affinity column chromatography (fig.3): 40–50% of the asialo GM1 was retained on the PNA–Sepharose column in contrast to only 10% of total GM1 bound in the column. This difference could be explained by postulating either that PNA preferentially recognizes the trisaccharide Gal(β1–3)GalNAc (β1–4)Gal over the disaccharide Gal(β1–3)GalNAc, or that the sialic acid residue of GM1 participates in the interaction with the *N*-acetyl group of GalNAc as suggested by NMR studies [32] and in hindering the binding of PNA with Gal(β1–3)GalNac.

PNA receptor- and asialo GM1-positive cell populations in rat thymus were compared: 5–10% of the total cells of rat thymus are asialo GM1 positive; they were present in the cortico-medullar junction [22]. However, thymocytes are known to be separated into cortical (immature) and medullar (mature) cells by their different PNA binding affinity [6]. Therefore, it is of interest to know whether asialo GM1-positive cells are PNA receptor-positive or not. Under conditions in which anti-asialo GM1 antibody lysed 20% of the total thymocytes, it lysed 31% of the PNA receptor-positive cells and 14% of the PNA receptor-negative cells (fig.4). Therefore, it appears that PNA receptor-positive and asialo GM1-positive cell populations overlap considerably.

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