A new phosphoglycerolipid, 'phosphatidylglucose', found in human cord red cells by multi-reactive monoclonal anti-i cold agglutinin, mAb GL-1/GL-2

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Abstract Cord red cell membranes express many differentiation-related molecules. To study such molecules, we have established human cell lines, termed GL-1 and GL-2, by the Epstein–Barr virus transformation method, both of which produce monoclonal anti-i cold agglutinin [Y. Nagatsuka et al., Immunol. Lett. 46 (1995) 93–100]. Thin layer chromatography immunoblotting analysis revealed that these antibodies had broad specificities reacting with a variety of glycolipid antigens. Of the immunoreactive lipid antigens, a new phosphoglycerolipid containing glucose from human cord red cells was found. The isolated lipid was unstable to alkaline hydrolysis and contained glucose as a sole sugar. Secondary ion mass spectrum–collision-induced dissociation mass spectrometric analysis of this lipid gave the main molecular ion peak at *m/z* 885 corresponding to phosphatidylhexose. This antigen was susceptible to phospholipases A2, C and D but resistant to phosphatidylinositol-specific phospholipase C. Two-dimensional nuclear magnetic resonance spectroscopy confirmed that glucose is linked to the *sn*-glycerol 3-phosphate residue with a β-anomeric configuration. Based upon these combined results, we identified this lipid as phosphatidyl-β-D-glucose. This is the first report showing the presence of the glucosylated glycerophospholipid in mammalian sources.

Key words: Epstein–Barr virus; Human monoclonal antibody; Cold agglutinin; Cord red cell; Glycolipid

1. Introduction

Natural autoantibodies generally consist of IgM immunoglobulins encoded by germline genes displaying 'multi-reactivity' with low affinity towards a wide range of antigens (for review, see [1]). Anti-i cold agglutinins belong to such natural autoantibodies mostly of the IgM class (for review, see [2]), which are encoded by the VH4-21 gene [3–5]. The i antigens include carbohydrate moieties of glycoproteins or glycolipids containing repeating *N*-acetyllactosamine units as its determinant [6–8]. However, the same IgM antibodies have been reported to be cross-reactive even with DNA and lipid A of Gram-negative bacteria [9,10]. Though the affinity to individual antigens is low, such antibodies are considered to play a physiological role in early defense mechanisms.

To study differentiation-related antigens, we previously established two human B-cell lines, GL-1 and GL-2, both producing anti-i antibodies (mAbs) [11]. By using the mAbs, we found that a glycolipid antigen expressed in an Epstein–Barr virus (EBV)-negative B-cell line is down-modulated by EBV infection [12]. Unexpectedly, we have found that the target antigen of the B-cell line was not an i-active glycolipid but an alkaline labile glycolipid possessing 9-*O*-acetylated sialic acid [12]. However, in the case of human cord red cells, during purification of antigenic lipids, we noticed the presence of another immunoreactive lipid with a similar behavior to phosphatidylinositol on thin layer chromatographic (TLC) analysis.

To determine the chemical structure of the mAb GL-1/GL-2-reactive antigen, we isolated the major antigenic lipid from cord red cells with monitoring by TLC immunostaining. Here we identify one of the major immunoreactive compounds as phosphatidylglucose.

2. Materials and methods

2.1. Monoclonal antibody

Human mAbs GL-1 and GL-2 were prepared with EBV transformation as previously described [11]. In this report, we mainly used mAb GL-2.

2.2. Extraction and partial purification of antigenic lipid from cord red cells

Human cord blood was collected under sterile conditions and immediately lyophilized because a preliminary result had shown that both red cells and serum contained the immunoreactive lipid though the contents in red cells were higher than in serum. We used whole cord blood in this experiment to obtain as much antigenic lipid as possible. The total lipid was extracted from the pooled lyophilized

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Abbreviations: EBV, Epstein–Barr virus; PLA, C and D, phospholipase A, C and D; PI-PLC, phosphatidylinositol-specific phospholipase C; TLC, thin layer chromatography; SIMS, secondary ion mass spectrum; CID, collision-induced dissociation; TOCSY, total correlation spectroscopy

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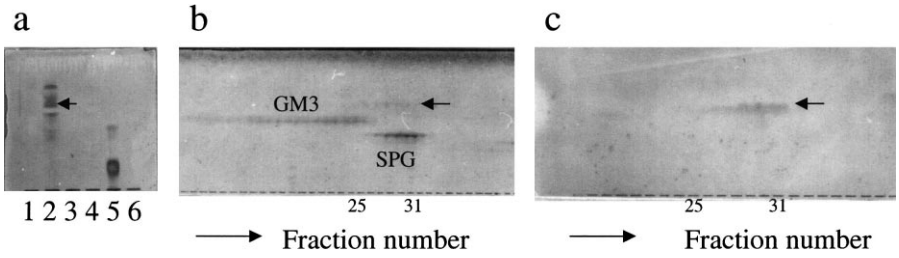

Fig. 1. a: TLC immunostaining of cord (lanes 1 and 2) and adult (lanes 3 and 4) red cell whole lipid extracts (erythrocyte volume 0.1 ml equivalent) with mAb GL-2. Lanes 1 and 3, PB-1; lanes 2 and 4, PB-2. Lane 5, standard i-ganglioside (prepared from bovine erythrocytes containing over 90% of i-ganglioside); lane 6, standard ganglioside mixture, containing GM3, GM2, GM1, GD1a, GD1b, GT1b. b: The major immunoreactive lipid (indicated by an arrow in the chromatogram) was eluted in the ganglioside fraction on a Q-Sepharose column. Spots were visualized by resorcinol–HCl followed by orcinol–H_2SO_4 reagent. c: The elution profile of immunoreactive lipid from the same column determined by TLC immunostaining. The immunoreactive fractions were pooled, dialyzed against water, and lyophilized, then used for further analysis.

powder (496 g) with chloroform (C):methanol (M) (2:1, v/v), twice, then C:M:water (W) (5:8:3, v/v), twice. The extracts were combined, evaporated, dialyzed against water, then lyophilized. Approximately one-fifth of the lyophilized extract obtained was applied onto a Q-Sepharose column (2.5×60 cm) which was pre-equilibrated with C:M:W (30:60:8, v/v). The column was washed with the same solvent to remove the neutral lipids, then Q-Sepharose-retained materials were eluted with a gradient from 200 ml C:M:W (30:60:8, v/v) to 200 ml C:M:1 M aqueous sodium acetate (30:60:8, v/v). Elution was monitored on TLC with resorcinol spray and TLC immunostaining with mAb GL-2. The immunoreactive fractions were pooled, evaporated and dialyzed against water, then lyophilized. The sample was treated with phosphatidylinositol-specific phospholipase C (PI-PLC) according to the method of Ikezawa et al. [13]. The PI-PLC digest was dialyzed against water, lyophilized, and dissolved in C:M (9:1, v/v), then loaded onto a phenylboronate agarose (PBA 60, Millipore Corporation, USA) column which was pre-equilibrated with C:M (9:1, v/v). The column was washed with five column volumes of C:M (9:1, v/v) and with a column volume of C:M (2:1, v/v) (PB-1). The PBA-retained materials were then eluted by five column volumes of C:M:W (5:5:1, v/v) (PB-2). The PB-2 fraction was dialyzed against water, then applied onto a Senshu-pack Aquasil HPLC column (Senshu-Kagaku, Japan) and the immunoreactive lipid was eluted with a gradient from C:M:W 60:20:0.5 (v/v) to C:M:W 60:25:1 (v/v). Elution was monitored by TLC immunostaining and the immunoreactive fraction was pooled.

2.3. TLC immunostaining

TLC immunostaining was performed according to the method of Higashi et al. [14,15] except that the 3,3′-diaminobenzidine tetrahydrochloride kit (ICN Biochemicals, Inc., USA) with sensitizing by the addition of 1 mM $NiCl_2$ was used as chromogenic substrate.

2.4. Two-dimensional TLC

The partially purified antigen was spotted onto the origin of the TLC plate Polygram Sil G (Macherey-Nagel, Germany). 2D TLC was developed twice with the following solvent mixtures for each direction. The TLC was developed with a solvent system of C:M:aqueous 12 mM $MgCl_2$ (60:35:8, v/v) as the first solvent, then it was developed with C:acetone (A):M:acetic acid (HOAc):W (3:4:1:1:0.5, v/v) [16] as the second solvent for the first dimension. After being well dried, it was developed with C:M:20% aqueous ammonia (60:35:8, v/v) as the first solvent and C:A:M:HOAc:W (3:4:1:1:0.5, v/v) as the second solvent for the second dimension.

2.5. TLC blotting and mass spectrometry

The immunoreactive lipid separated on the 2D TLC was transferred onto a PVDF membrane by the TLC blotting technique [17,18]. Then it was applied to negative secondary ion mass spectrometry (SIMS) by a TSQ 70 triple quadrupole mass spectrometer (Finnigan MAT, USA) [17,19].

2.6. Hexose analysis

The marked spot on the PVDF membrane blot was cut and extracted twice with a small volume of C:M (2:1, v/v). The extract was dried and then methanolized with 5% HCl–methanol at 80°C for 2 h. After nitrogen gas flushing, the sample was trimethylsilylated by the addition of 20 μl of trimethylsilylimidazole and 200 μl of pyridine, then applied to an OV-17 column equipped with a gas mass spectrometer (Finnigan-MAT, USA) and analyzed by raising the temperature from 100°C to 250°C at a rate of 5°C/min.

2.7. Nuclear magnetic resonance (NMR) spectrum

The immunoreactive lipid on the 2D TLC was scraped. The silica gel powder was packed into a small column (4×4 mm) and eluted with C:M (2:1, v/v) and C:M:W (5:5:1, v/v). After evaporation of the solvent by centrifugal vacuum evaporation, the lipid was dissolved into d_6-dimethyl sulfoxide and the 2D FG total correlation spectroscopy (TOCSY) spectrum was obtained by a JEOL α-600 NMR spectrometer.

2.8. Quantitative determination of phosphatidylglucose in cord red cells

The amount of phosphatidylglucose was calculated by phosphorus

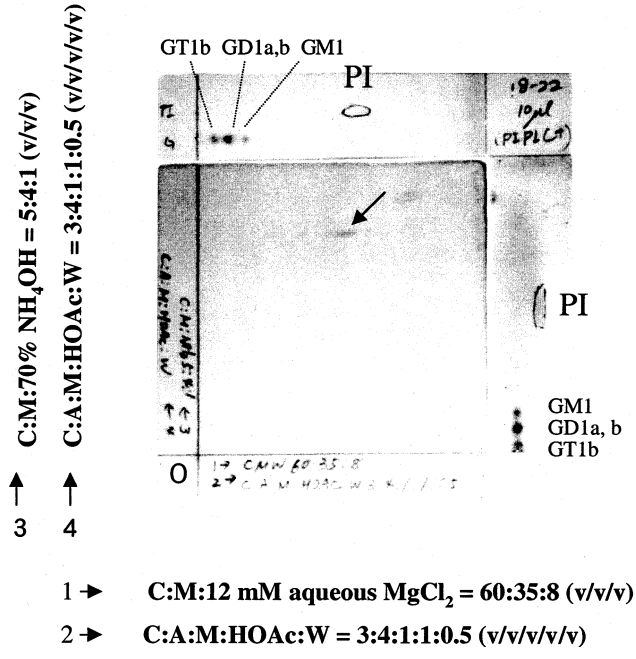

Fig. 2. Separation of the immunoreactive compound and PI on 2D TLC. A part of the Senshu-pack HPLC immunoreactive fraction was spotted onto the origin of the TLC plate and developed as described in Section 2. The standard lane was cut off and PI was detected under UV light after spraying pulimurin reagent. Standard gangliosides were visualized by resorcinol spray. The sample area was stained with mAb GL-2, and the immunoreactive spot was detected by peroxidase-conjugated anti-human Igs using Ni-sensitized diaminobenzidine substrate.

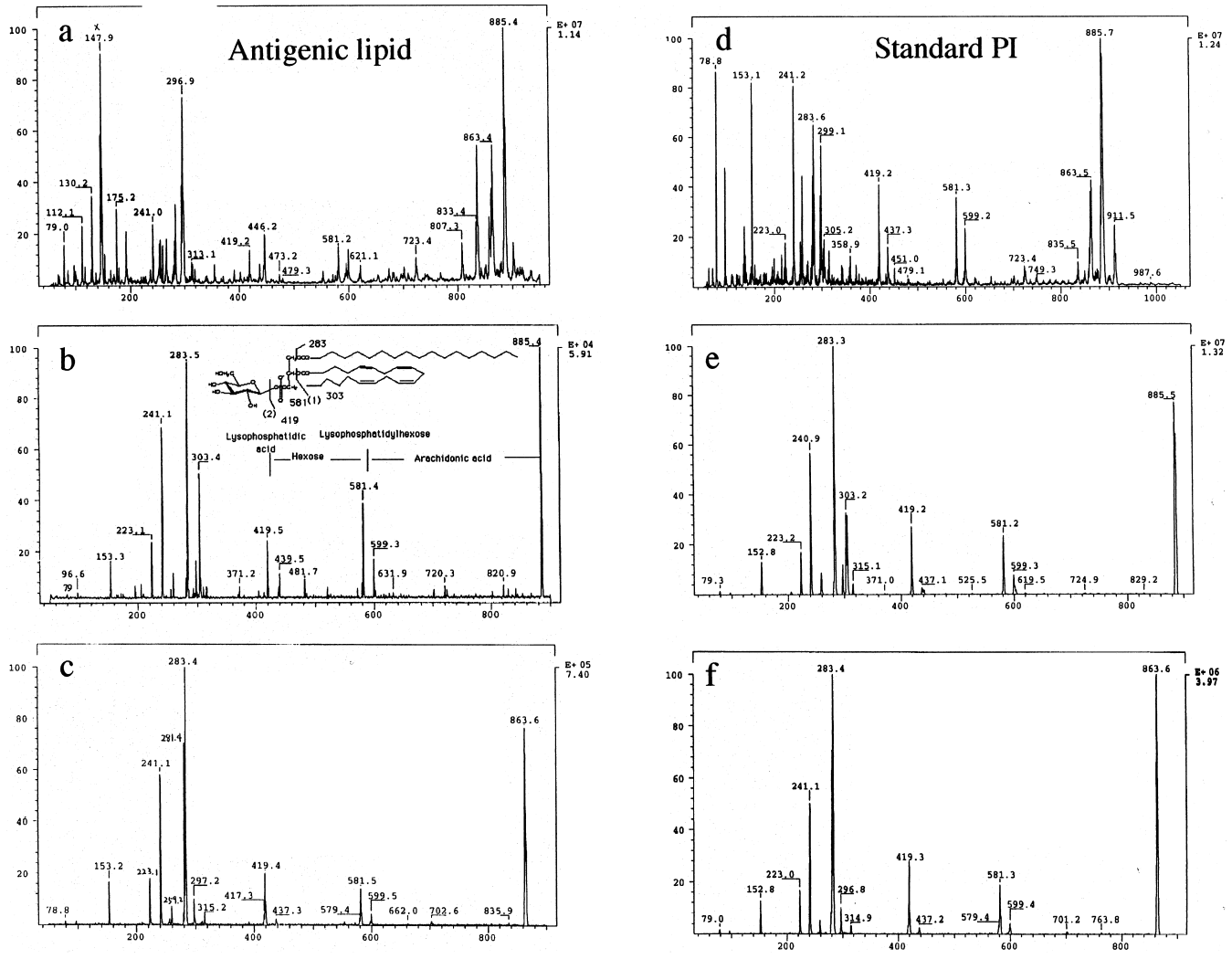

Fig. 3. Secondary ion mass spectra of the lipid purified form cord red cells (a–c) and PI (d–f). The immunoreactive lipid separated by 2D TLC was blotted onto a PVDF membrane and recovered from the blotted membrane. a,d: Total SIMS pattern. b,e and c,f: Spectra derived from *m/z* 885 and *m/z* 863, respectively.

content determined with the method of Bartlett [20] and glucose contents assayed by gas chromatography (described in Section 2.7).

3. Results

3.1. Occurrence of mAb GL-2 reactive lipid antigens in human cord red cells

We isolated the anti-i cold agglutinin, mAb GL-2, as described previously [11]. These mAbs agglutinate human cord red cells but not adult red cells on ice but not at room temperature. The minimal amount of IgM protein required for cord red cell agglutination was 12.5 ng. The mAbs immunostained several lipids in the PBA-retained fraction (PB-2) from cord red cells. The reactive lipids were greatly reduced in adult red cell lipids on TLC, as shown in Fig. 1a. The major immunoreactive lipid was partially purified on a Q-Sepharose column (Fig. 1b,c). The immunoreactive lipid (indicated by an arrow in the figure) was eluted slightly after the major ganglioside GM3 and slightly before sialylparagloboside. The immunoreactive fraction on Q-Sepharose was pooled and used as a starting material for further purification and structural analysis as described below.

3.2. Isolation and characterization of a major immunoreactive compound

To characterize the structure of immunoreactive lipids, we further purified a major immunoreactive compound from the Q-Sepharose fraction by PBA and Iatrobeads column chromatographies. The immunoreactive compound thus obtained showed a single spot on TLC determined with resorcinol followed by orcinol reagent. Then, the lipid was subjected to negative SIMS–collision-induced dissociation (CID) mass spectrometric analysis. A major molecular ion peak was observed at *m/z* 885 corresponding to phosphatidylinositol (Fig. 3) [21,22]. However, the antigenic lipid was insensitive to PI-PLC although it was susceptible to phospholipase (PL) A, C and D.

Since there was a possibility that PI was present in the Iatrobeads fraction, we removed the contaminant PI in the Q-Sepharose fraction by extensive treatment with PI-PLC. The resultant lipid fraction was purified by PBA and Senshu-pack Aquasil HPLC column chromatographies. However, the purified lipid gave the same molecular ion peak at *m/z* 885.

To further confirm that the immunoreactive lipid is different from PI, we developed a solvent system to separate PI and

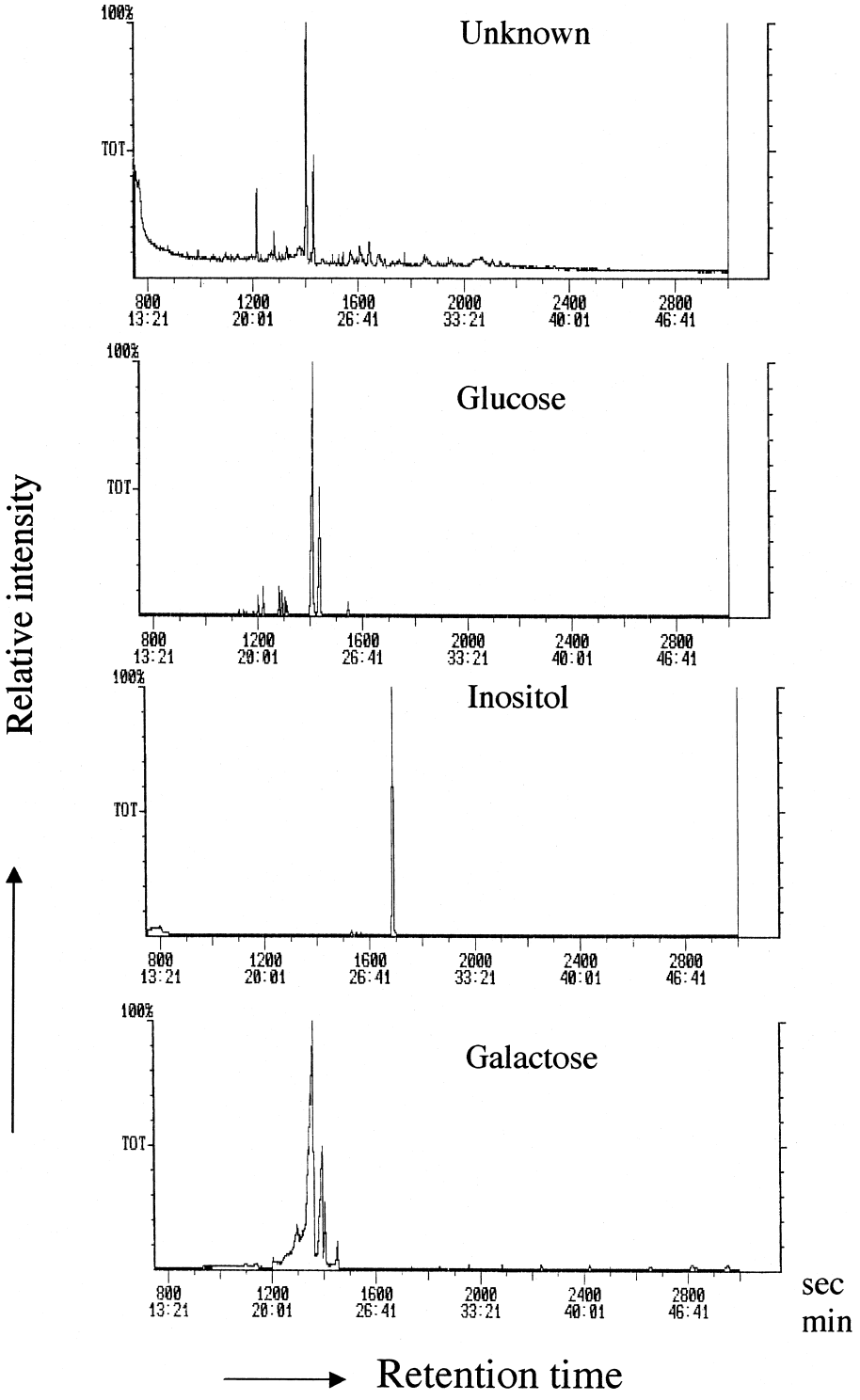

Fig. 4. Hexose analysis of the immunoreactive spot by GC-MS. The immunoreactive spot on the 2D TLC was blotted directly onto the PVDF membrane, and the blotted spot was cut off, extracted twice with C:M (2:1, v/v), evaporated, and then methanolized with 5% HCl–methanol at 80°C for 2 h. The obtained methylglycoside was trimethylsilylated with trimethylsilylimidazole and analyzed by gas chromatography. The methylglycosides of authentic glucose, galactose and inositol were similarly trimethylsilylated and used as standards.

the immunoreactive lipid on TLC. The immunoreactive fraction from Senshu-pack was analyzed by 2D TLC as described in Section 2. Fig. 2 shows clearly that the immunoreactive lipid moved faster than authentic PI in the second dimension.

3.3. SIMS with direct TLC blotting from immunostained TLC plates

To obtain the SIMS–CID spectrum of the immunoreactive spot itself, we blotted the spot shown in Fig. 2 directly onto

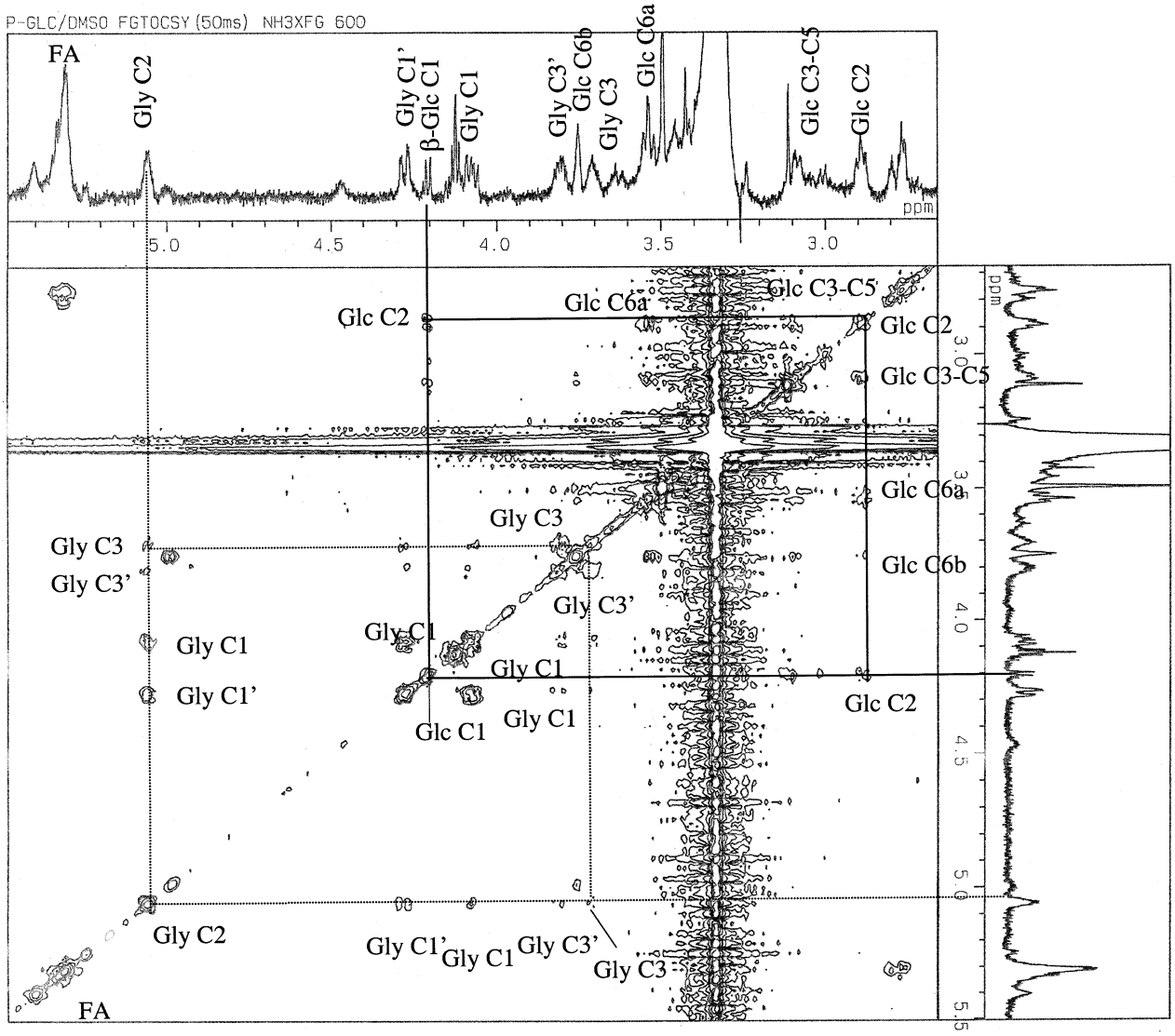

Fig. 5. 2D TOCSY spectrum of phosphatidylglucose. A 600 MHz TOCSY spectrum of phosphatidylglucose was measured in a solution of *d*-DMSO at 25°C, mixing time of 50 ms. In the spectrum, Glc, Gly and FA indicate protons on the β-Glc, glycerol and fatty acid double bonds, respectively. Approximately 150 μg of phosphatidylglucose was applied.

the PVDF membrane and analyzed it by SIMS–CID mass spectrometry. As shown in Fig. 3a, the most abundant negative molecular ion was again observed at *m/z* 885. The SIMS–CID spectra of *m/z* 863 and 885 are also shown in Fig. 3b,c. Both molecules gave a fragment ion at *m/z* 581 corresponding to its lyso-species. A fragment ion at *m/z* 419 was lysophosphatidic acid. A fragment ion at *m/z* 283 was fatty acid, C18:0 at the C1 position. Fatty acids at the C2 position were observed at *m/z* 281 and 303 corresponding to C18:1 and C20:4 derived from $[M-H]^-$ 863 and 885, respectively. Hexose phosphate and hexose phosphate minus H_2O were observed at *m/z* 241 and 223, respectively. The presence of PO_3^- and $H_2PO_4^-$ was confirmed by fragment ions at *m/z* 79 and 97. The SIMS–CID spectra of the immunoreactive lipid are very similar to that of authentic PI, as shown in Fig. 3d–f. Thus, the immunoreactive lipid has the same backbone structure as PI and contained hexose instead of inositol.

Table 1
Chemical shift and coupling constant of glucose and glycerol of phosphatidylglucose

Position	Chemical shift (ppm)	Coupling (Hz)
Glucose C1	4.20	d (8.4)
Glucose C2	2.89	t (7.5)
Glucose C3, 4 and 5	3.08	ND
Glucose C6a	3.54	t (7.6)
Glucose C6b	3.75	ND
Glycerol C1	4.06	q (6.7)
Glycerol C1′	4.27	q (9.0; 3.0)
Glycerol C2	5.05	ND
Glycerol C3	3.70	ND
Glycerol C3′	3.81	q (4.6)

ND: not defined. Chemical shifts of the C1–C6 protons of glucose and the C1–C3 protons of glycerol were determined from the 2D TOCSY spectrum with a mixing time of 50 ms.

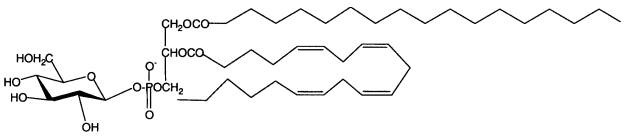

Fig. 6. Proposed structure of the major phosphatidylglucose.

3.4. The immunoreactive compound contains glucose but not inositol as hexose

To determine the hexose species, the immunoreactive compound was extracted from the blots and analyzed as described in Section 2. The immunoreactive compound contained glucose, but neither inositol nor galactose (Fig. 4). This result indicates that the immunoreactive compound is a phosphoglycerolipid containing glucose linked to phosphatidic acid, that is 'phosphatidylglucose'.

3.5. NMR spectrum of the immunoreactive lipid

For further confirmation of the structure of phosphatidylglucose, the lipid as subjected to NMR 2D TOCSY analysis. An anomeric proton of glucose was assigned at $\delta = 4.2$ ppm and a coupling constant $J_{1,2}$ value of 8.4 Hz revealed that the antigenic molecule contains glucose with a β-anomeric configuration (Fig. 5 and Table 1) whereas the standard α-D-glucose-1-phosphate showed an anomeric proton at $\delta = 5.45$ ppm and a coupling constant $J_{1,2}$ value of 7.6 and 4.0 Hz. The PI C1 proton has been reported as $\delta = 3.9$ ppm and a $J_{1,2}$ value of 2.7 Hz [23]. Combining all these results, one of the major cord red cell lipid antigens reacting with mAb GL-1/GL-2 was identified as phosphatidyl β-D-glucose (Fig. 6).

3.6. Level of phosphatidylglucose in cord red cells

The amount of phosphatidylglucose in cord red cells was estimated based upon phosphorus analysis of immunostained TLC. The approximate amount of phosphatidylglucose was estimated to be 10 nmol/g dried red cells, determined by the phosphorus contained in the immunoreactive spot, whereas the amount of phosphatidylinositol was approximately 3 nmol. The yields of glucose and phosphorus in the final product were 2.2 and 3.1 nmol/g dried red cells (molar ratio 1:1.4), respectively.

4. Discussion

Natural antibodies are often multi-reactive. Affinities to individual antigens are generally low but they recognize a wide range of antigens. For example, Bohn et al. [24] obtained human mAbs, which bind to tumor cells to inhibit cell growth, from a non-tumor-associated patient. This mAb also reacts with tetanus toxin or DNA, which is not expressed on the surface of tumor cells. Such antibodies have been disregarded because their specificities were very low and accordingly they were not applicable as immunobiochemical reagents. However, Avrameas [25] and Coutinho et al. [1] described the biological significance of natural autoantibodies. They play roles (1) in removal of senescent/altered self components, (2) as the first line of natural defense against infectious agents, (3) as immune regulatory factors through idiotypic networks, and (4) as a platform for antigen-specific immune responses from which strictly specific 'immune antibodies' are derived by selective pressure of foreign antigens.

'Phosphatidylglucose' has been reported in a few microbes. Smith and Henrickson [26] have reported a glucose-containing phospholipid in *Mycoplasma laidlawii* strain B whose infrared spectrum possessed some resemblance to that of PI and they reported it as phosphatidylglucose. However, this lipid was resistant to PLA, PLC and PLD digestion. Later, Shaw et al. [27] proposed that the *M. laidlawii* lipid is not phosphatidylglucose but glycerylphosphoryldiglucosyl diglyceride. Short and White [28] isolated two glucose-containing phospholipids from *Staphylococcus aureus* and identified one of them as phosphatidylglucose, which was sensitive to PLC and PLD.

The possibility that phosphatidylglucose is derived from bacterial contamination in cord blood is unlikely because the cord blood was taken under sterile conditions and immediately frozen before use. In addition, phosphatidylglucose was always detected when the lipid was prepared and analyzed from each small-scale sample. Furthermore, no bacterial endotoxin could be detected in the crude extract, Q-Sepharose fraction or Senshu-pack HPLC fraction.

Up to now, glucosylceramide and sterol glucoside have been reported to exist as glucosylated forms of lipids in sphingolipid and cholesterol, respectively. Interestingly, they play physiologically important roles in eukaryotic cell membranes [29–33]. For example, Yamashita et al. [33] demonstrated that disruption of glucosylceramide synthase halts embryonic differentiation by the gene targeting technique. It is expected that the new lipid described here might have potential roles in mammalian cells, too. To understand the biological roles of this lipid, further studies will be needed. For example, we observed that mAb GL-1/GL-2 treatment stimulated cell growth of BJA-B cells and cell differentiation of HL60 cells (unpublished results). If this newly found lipid is present on the outer leaflet of the cell surface membrane, it can be expected that phosphatidylglucose may play a role in the biological processes.

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References

[1] Coutinho, A., Kazatchkine, M.D. and Avrameas, S. (1995) Curr. Opin. Immunol. 7, 812–818.
[2] Pruzanski, W. and Katz, A. (1984) Clin. Immunol. Rev. 3, 131–168.
[3] Pascual, V., Victor, K., Spellerberg, M., Hamblin, T., Stevenson, F.K. and Capra, J.D. (1992) J. Immunol. 149, 2237–2344.
[4] Chapman, C.J., Spellerberg, M.B., Smith, G., Carter, S.J., Hamblin, T.J. and Stevenson, F.K. (1993) J. Immunol. 151, 1051–1061.
[5] Smith, G., Spellberg, M., Boulton, F., Roelcke, D. and Stevenson, F. (1995) Vox Sang. 68, 231–235.
[6] Feizi, T. and Childs, R.A. (1978) Biochem. J. 173, 245–254.
[7] Gooi, H.C., Veyrières, A., Alais, J., Scudder, P., Hounsell, E.F. and Feizi, T. (1984) Mol. Immunol. 21, 1099–1104.
[8] Okada, Y., Kannagi, R., Levery, S.B. and Hakomori, S.-I. (1984) J. Immunol. 133, 835–842.
[9] Bhat, N.M., Bieber, M.M., Chapman, C.J., Stevenson, F.K. and Teng, N.N.H. (1993) J. Immunol. 151, 5011–5021.
[10] Spellerberg, M.B., Chapman, C.J., Mockridge, C.I., Isenberg, D.A. and Stevenson, F.K. (1995) Hum. Antibodies Hybridomas 6, 52–56.
[11] Nagatsuka, Y., Watarai, S., Yasuda, T., Higashi, H., Yamagata, T. and Ono, Y. (1995) Immunol. Lett. 46, 93–100.

[12] Nagatsuka, Y., Yamaki, M., Watarai, S., Yasuda, T., Higashi, H., Yamagata, T. and Ono, Y. (1996) Virus Res. 43, 57–68.
[13] Ikezawa, H., Yamanegi, M., Taguchi, R., Miyashita, T. and Ohyabu, T. (1976) Biochim. Biophys. Acta 450, 154–164.
[14] Higashi, H., Hirabayashi, Y., Fukui, Y., Naiki, M., Matsumoto, M., Ueda, S. and Kato, S. (1985) Cancer Res. 45, 3796–3802.
[15] Higashi, H., Fukui, Y., Ueda, S., Kato, S., Hirabayashi, Y., Matsumoto, M. and Naiki, M. (1984) J. Biochem. 95, 1517–1520.
[16] Nakajima, S., Nagata, K., Banno, Y., Sakiyama, T., Kitagawa, T., Miyawaki, S. and Nozawa, Y. (1984) J. Lipid Res. 25, 219–227.
[17] Taki, T., Kasama, T., Handa, S. and Ishikawa, D. (1994) Anal. Biochem. 223, 232–238.
[18] Taki, T., Ishikawa, D., Handa, S. and Kasama, T. (1995) Anal. Biochem. 225, 24–27.
[19] Kasama, T., Hisano, H., Nakajima, M., Handa, S. and Taki, T. (1996) Glycoconjug. J. 13, 461–469.
[20] Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468.
[21] van Breemen, R.B., Wheeler, J.J. and Boss, W.F. (1990) Lipids 25, 328–334.
[22] Jensen, N.J., Tomer, K.B. and Gross, M.L. (1987) Lipids 22, 480–489.
[23] Shibata, T., Uzawa, J., Sugiura, Y., Hayashi, K. and Takizawa, T. (1984) Chem. Phys. Lipids 34, 107–113.
[24] Bohn, J., Niemann, B., Roggenbuck, D., Docke, W. and Jahn, S. (1995) Clin. Exp. Immunol. 99, 376–383.
[25] Avrameas, S. (1991) Immunol. Today 12, 154–159.
[26] Smith, P.F. and Henrikson, C.V. (1965) J. Lipid Res. 6, 106–111.
[27] Shaw, N., Smith, P.F. and Verheij, H.M. (1970) Biochem. J. 120, 439–441.
[28] Short and White (1970) J. Bacteriol. 104, 126–132.
[29] Muhiudeen, I.A., Koerner, T.A.W., Samuelsson, B., Hirabayashi, Y., DeGasperi, R., Li, S.-C. and Li, Y.-T. (1984) J. Lipid Res. 25, 1117–1123.
[30] Wertz, P.W., Stover, P.M., Abraham, W. and Dowing, D.T. (1986) J. Lipid Res. 27, 427–435.
[31] Abraham, W., Wertz, P.W., Burken, R.R. and Downing, D.T. (1987) J. Lipid Res. 28, 446–449.
[32] Hungund, B.L., Dayal, B., Dayal, V.K. and Salen, G. (1994) Chem. Phys. Lipids 69, 163–173.
[33] Yamashita, T., Wada, R., Sasaki, T., Deng, C., Bierfreund, U., Sandhoff, K. and Prola, R. (1999) Proc. Natl. Acad. Sci. USA 96, 9142–9147.