GLYCOLIPID COMPOSITION OF BLOOD GROUP P ERYTHROCYTES

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1. Introduction

The P blood-group system of human erythrocytes is made up of five phenotypes P_1, P_2, p, p_1^k, and p_2^k, which can be differentiated with three antibodies: anti-P, anti-P_1 and anti-p^k (see [1]).

These antibodies react with the corresponding antigens at the erythrocyte surface, but the specificity of anti-p^k and anti-P_1 is sometimes overlapping. The two common P_1 and P_2 phenotypes are defined by the presence on erythrocytes of the combination of P + P_1 antigen and of the P antigen alone, respectively. The three remaining phenotypes are very rare. In p phenotype all three P antigens are missing. Erythrocytes of the p_1^k blood group contain p^k and P_1 antigens, whereas only the former antigen is present in p_2^k cells.

The probable chemistry of P antigens has been elucidated only recently. Cory et al. [2] isolated the determinant of P_1-active glycoprotein from hydatid cyst fluid and characterized it as α-D-galactopyranosyl-(1→4)-D-galactose. Naiki and Marcus [3] found that ceramide trihexoside and globoside, the common cell-membrane glycolipids, displayed significant p^k and P blood-group activity, respectively. More recently, Marcus et al. [4] isolated from erythrocyte membranes a P_1-active ceramide pentasaccharide (for structures see table 1). On the basis of these findings Naiki and Marcus [3] predicted that individuals with p phenotype should be unable to synthesize ceramide

Table 1
Structures of erythrocyte glycolipids referred to in the paper

(1) Lactosylceramide	βGal(1→4)βGlcCer
(2) Ceramide trihexoside p^k-antigen	αGal(1→4)βGal(1→4)βGlcCer
(3) Globoside P-antigen	βGalNAc(1→3)αGal(1→4)βGal(1→4)βGlcCer
(4) Lacto-*N*-neotetraosylceramide[a]	βGal(1→4)βGlcNAc(1→3)βGal(1→4)βGlcCer
(5) Glycolipid with P_1 activity	αGal(1→4)βGal(1→4)βGlcNAc(1→3)βGal(1→4)βGlcCer
(6) Fucose-containing glycolipid with H activity	βGal(1→4)βGlcNAc(1→3)βGal(1→4)βGlcCer ↑(1→2) αFuc
(7) Sialic acid-containing glycolipid	βGal(1→4)βGlcNAc(1→3)βGal(1→4)βGlcCer ↑(2→3) αNANA

[a] The simplest GlcNAc-containing glycolipid found in human erythrocyte membranes.
The abbreviations used are: Gal, galactose; Glc, glucose; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; Fuc, fucose; NANA, *N*-acetylneuraminic acid.

trihexoside, globoside and P_1-active glycolipid. The present communication supports this prediction because both ceramide trihexoside and globoside were found to be missing from p erythrocytes. In addition we provide evidence for the accumulation in p erythrocytes of lactosylceramide and of glycolipids containing sialic acid and *N*-acetylglucosamine.

2. Materials and methods

A blood sample of group ORh(−)p was collected in Sweden. Other blood samples used in this study were obtained from blood donors in Warsaw.

Standard preparations of glucosylceramide, lactosylceramide, ceramide trihexoside and globoside were isolated from human erythrocyte membranes by the usual techniques [10]. Glycolipid with the structure: βGlcNAc(1→3)βGal(1→4)βGlcCer was obtained by partial acid hydrolysis of lacto-*N*-neotetraosyl ceramide. The latter was supplied by Mr T. Pacuszka.

Ghosts for the analysis of lipid carbohydrates were prepared by the method of Dodge et al. [5]. The freeze-dried ghosts from 1.5 ml of packed erythrocytes were extracted with 1 ml portions of chloroform–methanol 2:1 solvent mixture. The pooled extracts were filtered through glass wool and partitioned according to Folch et al [6]. For thin-layer separation of glycolipids the extraction was performed on acetone powders prepared from 2 ml portions of packed erythrocytes. The extracts were evaporated to dryness and hydrolysed with 1 M KOH for 24 h at 37°C. After neutralization the hydrolysates were dialysed and freeze-dried. The residues were separately dissolved in chloroform–methanol 2:1, filtered through glass wool and then chromatographed on 7 × 1.4 cm columns packed with silicic acid in chloroform. The columns were eluted first with chloroform (25 ml) and next with 25 ml of the lower phase of chloroform–methanol–water (65:30:8). The orcinol-positive materials eluted with the second solvent were collected and analysed under conditions specified in the legend to fig.1.

The carbohydrate composition of lipid extracts was determined by gas-liquid chromatography. Analyses were performed with a GCV Pye Unicam apparatus equipped with a flame ionization detector and a DP-80 digital integrator. Alditol and hexosaminitol acetates were analysed on 200 × 0.4 cm glass columns packed with 1% OV-225 on chromosorb-Q. The carrier gas was nitrogen at 30 ml/min. The column was operated isothermally at 194°C until the glucitol acetate peak emerged. Thereafter the temperature was raised to 225°C to allow the release of hexosaminitols. Alditol acetates were additionally analysed on columns packed with 3% ECNSS-M on gas-chrom Q at 180°C. Sialic acid was determined by the method of Aminoff [8]. Prior to the determination it was purified by ion-exchange chromatography according to Spiro [9].

3. Results

Fig.1 shows a thin-layer separation of glycolipids derived from erythrocytes of the p, P_1 and P_2 blood groups. Ceramide trihexoside and globoside are clearly missing from p cells. Another evident abnormality of p erythrocytes is a large accumulation of lactosylceramide. Below the doublet of lactosylceramide a band is seen, which is not present in a significant amount in glycolipids derived from P_1 and P_2 cells. The mobility of this band does not correspond to any known glycolipid, including that with the structure βGlcNAc(1→3)βGal(1→4)βGlcCer. The chromatogram of p glycolipids also reveals some accumulation of complex glycolipids with low chromatographic mobilities, probably containing fucose, sialic acid and *N*-acetylglucosamine residues. The conclusions are supported by the analysis of the carbohydrate composition of the lipid extracts (see table 2). *N*-Acetylgalactosamine, a constituent of globoside and of A-active glycolipids, is practically not present in Op erythrocytes. Sialic acid and *N*-acetylglucosamine levels were increased.

4. Discussion

The unusual glycolipid composition of p erythrocytes, namely the absence of ceramide trihexoside and globoside, and the accumulation of lactosylceramide and of glycolipid containing sialic acid and *N*-acetylglucosamine may be best explained on the assumption that p phenotype results from a block in the synthesis

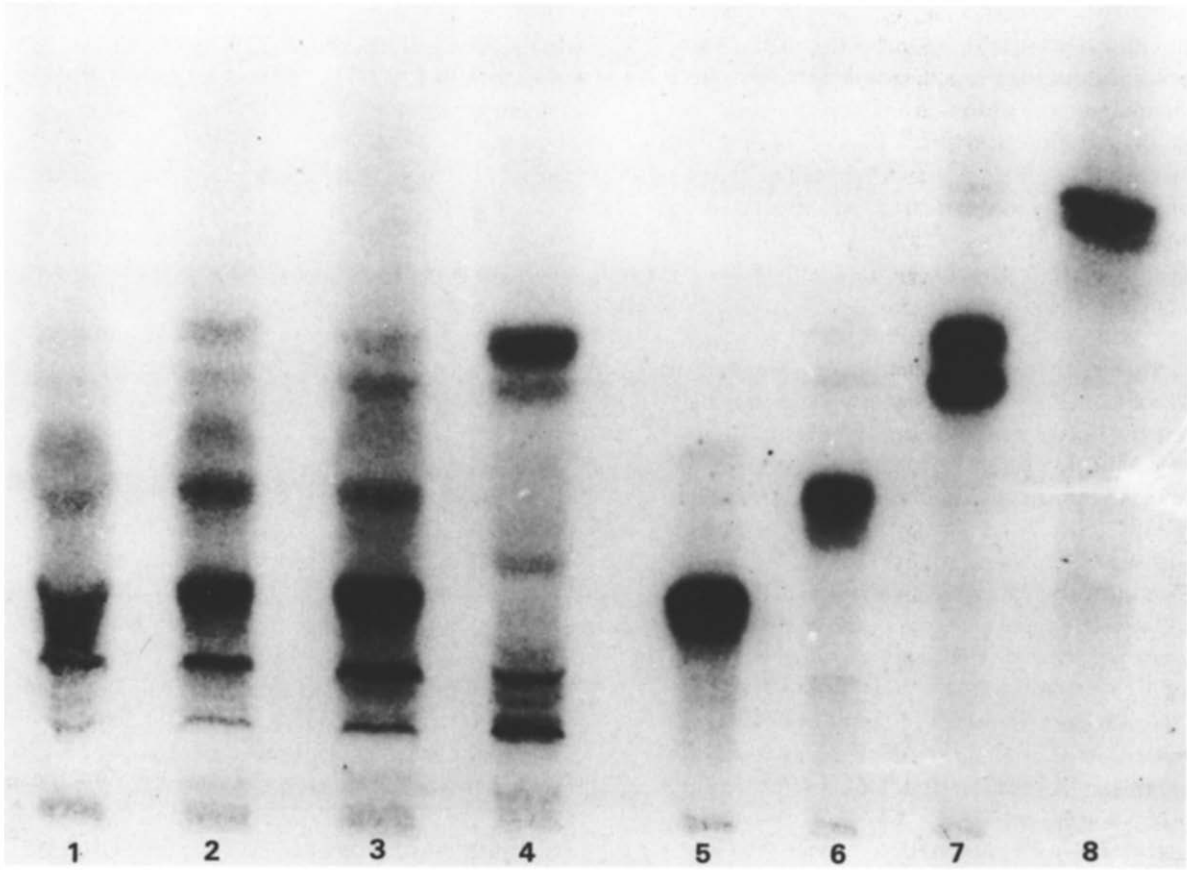

Fig.1. Thin-layer chromatogram of the glycolipids isolated from group O erythrocytes with various P blood-group specificaties: (1,2) glycolipids from two different samples of P_1 erythrocytes; (3) P_2 glycolipids; (4) p glycolipids; (5) standard globoside; (6) ceramide trihexoside; (7) lactosylceramide; (8) glucosylceramide. The chromatogram was developed on a thin-layer plate precoated with silica gel (Woelm). The solvent was the lower layer of the chloroform–methanol–water (65:30:8, v/v mixture). Spots were visualized by spraying the plates with 0.5% orcinol in 1 M ethanolic H_2SO_4 and heating at 105°C for 5 min.

Table 2
The carbohydrate composition of glycolipid fractions isolated from group O erythrocytes with P_1, P_2 and p blood-group specificities

Red cells	Glucose	Galactose	GalNAc	GlcNAc	Sialic acid
	(nmol/ml of packed erythrocytes)				
p	143	209	4	36	12.2
P_1	158	353	83	6	3.7
P_1	237	435	139	12	4.8
P_1	188	365	110	22	3.4
P_2	158	313	139	10	5.7

Sialic acid was estimated in the materials soluble in the upper phase obtained after partitioning of lipid extracts according to Folch et al. [6]. Other carbohydrates were determined in the materials soluble in the lower phase.
P_1 glycolipids were obtained from samples of blood of three different individuals.

of ceramide trihexoside, the natural precursor of globoside. Thus it is likely that the transferase which adds an α-galactosyl residue in 1→4-linkage to the galactose of lactosylceramide is inoperative in p individuals. The ineffectiveness of this transferase is probably responsible also for the observed lack of expression of P_1 antigen in p cells, although the exact relationship between the transferases synthesizing p^k and P_1 structures remains to be established. Genes responsible for the synthesis of these structures seem not to be allelic (see, [1]). Nevertheless it is clear from table 1 that the p^k and P_1 antigens are terminated with an identical disaccharide sequence. The accumulation of lactosylceramide in p erythrocytes is obviously due to the lack of any conversion of this glycolipid into ceramide trihexoside. The greater availability of lactosylceramide probably results in the increased synthesis of complex glycolipids which are lactosylceramide derivatives. These are glycolipids containing fucose, sialic acid and *N*-acetylglucosamine. In the present study the fucose content of p erythrocytes could not be measured with a sufficient degree of accuracy. However, since the sialic acid and fucose-containing glycolipids are structurally derived from the same *N*-acetylglucosamine-containing precursors, it is reasonable to suppose that fucose in the lipid extracts of p erythrocytes is also increased. Generally fucose-containing glycolipids of human erythrocyte membranes display blood-group A,B,H activity [10–12]. Therefore it is tempting to speculate that the expression of these antigens is increased in p erythrocytes. The mechanism for the phenotypic interaction between ABO and P blood group systems would be not through the linkage between genes but through the competition between unrelated transferases for a limited amount of a glycolipid acceptor.

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