HUMAN ERYTHROCYTE P AND P^k BLOOD GROUP ANTIGENS: IDENTIFICATION AS GLYCOSPHINGOLIPIDS

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Summary - The human erythrocyte P blood group system consists of three known antigens, P_1, P and P^k. We have identified the P antigen as the glycosphingolipid globoside, βGalNAc(1→3)αGal(1→4)βGal(1→4)Glc-cer, and the P^k antigen as ceramide trihexoside, αGal(1→4)βGal(1→4)Glc-cer. These data suggest, in contrast to previous hypotheses, that the P^k antigen is a biosynthetic precursor of P, and that neither P nor P^k is a precursor of P_1. These findings also provide an explanation for the apparent recessive inheritance of the P^k antigen, and for the nature of the biochemical abnormality in individuals of the rare P^k and p phenotypes.

The human erythrocyte P blood group system consists of three antigens, P, P_1 and P^k, and five phenotypes (Table 1) (1-5). The genetic and immunochemical relationships among these antigens are unclear, but it was suggested (5) that the products of the P_2 and P_1 genes act in sequence to convert a precursor substance to the P and P_1 antigens. The P^k locus is not linked to P_1 or P_2, and P^k is unusual among blood group antigens in not being inherited as a dominant character (4-6).

Immunochemical studies of a cross-reacting glycoprotein (7) isolated from hydatid cyst fluid demonstrated that both the P_1 and P^k antigens have carbohydrate determinants with an immunodominant terminal non-reducing α-galactosyl residue (8-10). These data, and the ability of P_1 erythrocytes to absorb some anti-P^k antibodies from anti-P_1PP^k sera, indicate some degree of immunological similarity between the P_1 and P^k antigens. The P antigen does not cross-react with either P_1 or P^k. We have previously extracted the P_1 antigen from

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Abbreviations: Gal = D-Galactose; Glc = D-Glucose; GalNAc = N-acetyl-D-galactosamine; GlcNAc = N-acetyl-D-glucosamine; NAN = N-acetylneuraminic acid, Ceramide = N-acylsphingosine; PBS, phosphate-buffered saline, 0.02 M potassium phosphate, pH 7.3, 0.15 M NaCl.

Table 1. The P Blood Group System

Phenotype	Frequency	Antigens on Erythrocytes	Antibodies in Serum
P_1	75%	P_1, P	none
P_2	25%	P	anti-P_1
p	very rare	none	anti-P_1PP^{k*}
p^k_1	very rare	P_1, P^k	anti-P
p^k_2	very rare	P^k	anti-P

*These sera agglutinate erythrocytes of all phenotypes except p, but they do not necessarily contain three separable monospecific antibodies, i.e., specific anti-P^k reagents can be made by absorption of some sera with P_1 erythrocytes but anti-P^k and anti-P_1 antibodies are removed from other sera by this procedure.

Table 2. Structures of Glycosphingolipids Used in This Study

CTH	αGal (1→4) βGal (1→4) Glc-Cer
Globoside	βGalNAc (1→3) αGal (1→4) βGal (1→4) Glc-Cer
Paragloboside	βGal (1→4) βGlcNAC (1→3) βGal (1→4) Glc-Cer
Ganglioside	αNAN (2→3) βGal (1→4) βGlcNAc (1→3) βGal (1→4) Glc-Cer

erythrocyte stroma and identified it as a glycosphingolipid (11), and the P antigen has also been detected in a lipid extract of erythrocyte stroma (12,13). Our recent studies of a pure P_1 glycolipid (14,15) demonstrated that a terminal non-reducing α-galactosyl residue is essential for P_1 activity, and that this glycolipid contains galactose, N-acetylglucosamine and glucose in a ratio of 3:1:1. We now report the identification of the P and P^k antigens as the glycosphingolipids globoside (Table 2) and ceramide trihexoside (CTH).

Materials and Methods

Globoside (16,17), CTH (18-20), the P_1 glycolipid (14) and the ganglioside (21-24) were isolated from human erythrocyte stroma. Paragloboside (24-26) was prepared by treatment of the ganglioside with neuraminidase (EC 3.2.1.18) from Cl. perfringens (Sigma Chem. Co., type VI), and isolation of the neutral glycolipid by chromatography on a column of silicic acid. The terminal α-galactosyl residue of the P_1 glycolipid was removed by treatment with fig α-galactosidase (EC 3.2.1.22) (27) obtained from Drs. S.-C. and Y.-T Li (Tulane University), and the α-galactosidase-treated P_1 glycolipid was isolated by preparative thin layer chromatography. A glycoprotein with P_1 and P^k antigenic activities was isolated from sheep liver hydatid cyst fluid by the method of Morgan and Watkins (8). Agglutination of P_1 erythrocytes by four hemagglutinating units of anti-P_1 antibody was completely inhibited by 40 ng/ml of the P_1 glycolipid, and by 600 ng/ml of the hydatid cyst glycoprotein (15).

Anti-P sera from individuals of the P^k phenotype were obtained from Dr. P. Tippett (Blood Group Reference Laboratory, London), Dr. D. J. Anstee (South West Regional Blood Transfusion Centre, Bristol) and Mr. W. L. Marsh (New York Blood Center). Sera from p individuals containing anti-P_1PP^k were obtained from Dr. P. Levine (Ortho Research Foundation, Raritan, N.J.) and Haut serum was obtained from Dr. P. Tippett. A specific anti-P^k serum was obtained by absorption of Haut serum with type B, P_1 erythrocytes. Samples of type O, p, and type O, P_1^k erythrocytes were provided by Mr. W. L. Marsh.

Hemagglutination tests were performed at 4° in microtiter plates with a 2% suspension of ficin-treated erythrocytes as described previously (28). For inhibition studies 0.025 ml of serum containing four hemagglutinating units was incubated with an equal volume of inhibitor for 12 hours at 4°, 0.025 ml of 2% ficin-treated erythrocytes was added, and hemagglutination patterns were read after an additional two hours at 4°. Glycolipid dispersions were prepared for the inhibition tests by evaporation of organic solvents

under a stream of nitrogen and dispersion in PBS containing five parts by weight of sodium taurocholate (Nutritional Biochemical Co.) for each part of glycolipid. Sodium taurocholate in PBS did not inhibit hemagglutination in concentrations up to 0.25 mg/ml. Before use the glycolipid dispersions were subjected to ultra-sonic irradiation for one minute in an ultra-sonic cleaning bath (Heat Systems Ultrasonics, Inc.).

Results and Discussion

The three anti-P sera were specifically inhibited by globoside and not by any of the other glycolipids listed in Table 3, or by hydatid cyst glycoprotein. Additional evidence for the identification of globoside as the P antigen is that an antiserum prepared against globoside (29) agglutinated ficin-treated P_1 and P_2 erythrocytes, but not p or P^k cells (15).

Agglutination of P_1^k erythrocytes by unabsorbed anti-P_1PP^k sera was inhibited by hydatid cyst glycoprotein, CTH and the P_1 glycolipid (Table 3). After removal of antibodies that cross-react with P_1 from Haut serum, by absorption with P_1 erythrocytes, the specific anti-P^k antibodies remaining were inhibited only by CTH. CTH does not inhibit the anti-P_1 sera that we have examined. These data support the suggestion of Voak et al. (10) that the P^k determinant has a terminal non-reducing α-galactosyl residue, and define more clearly the immunochemical difference between the P_1 and P^k antigens.

These findings clarify the relationship between the P_1, P and P^k antigens, and suggest the nature of the genetic abnormality underlying the P^k phenotype. The P^k antigen, CTH, appears to be the biosynthetic precursor of the P antigen, globoside. It seems likely that P^k individuals are unable to add N-acetyl-galactosamine to CTH to convert it into globoside, and consequently have a much larger quantity of CTH on the surface of their erythrocytes than normal individuals. Globoside is the major neutral glycosphingolipid of human erythrocytes, and estimates of the relative abundance of globoside to CTH in erythrocytes range from 6:1 (30) to 30:1 (25); our data are more in accord with the latter figure (24,25). The failure of normal P_1 and P_2 erythrocytes to be

Table 3. Inhibition of Anti-P_1 and Anti-P^k Antisera by Glycosphingolipids and Hydatid Cyst Glycoprotein

		P_2 erythrocytes			P^k_1 erythrocytes				
		Anti-P Sera			Anti-P_1P^k sera				Anti-P^k serum
Antigen	Highest conc. used for test	I	II	III	IV	V	VI	Haut.	Absorbed Haut[1]
	(μg/ml)	Lowest concentration of antigen that completely inhibits hemagglutination							
CTH	50	-*	-	-	3	1.5	0.4	3	6
Globoside	50	3	1.5	6	-	-	-	-	-
Paragloboside	5	-	-	-	-	-	-	-	-
Ganglioside	5	-	-	-	-	-	-	-	-
P_1 glycolipid	5	-	-	-	1.2	1.2	0.3	2.5	-
α-Galactosidase-treated P_1 glycolipid	5	-	-	-	-	-	-	-	-
Hydatid cyst P_1 glycoprotein	50	-	-	-	1.5	-	6	25	-

[1]Haut serum absorbed with B P_1 erythrocytes

*-indicates no inhibition

agglutinated by anti-P^k, despite the presence of appreciable quantities of CTH in these cells, may result from steric obstruction of CTH by the longer carbohydrate chains of the more abundant globoside molecules. The apparent recessive nature of the P^k antigen results from conversion of most of CTH to globoside.

Individuals of the p phenotype are apparently unable to synthesize globoside, CTH or the P_1 glycolipid. The differences in chemical composition between the P_1 antigen and the other two glycolipids indicate that neither P nor P^k is a biosynthetic precursor of P_1. It is not known whether the same α-galactosyl transferase adds the terminal sugar residue to the P_1 and P^k antigens or if two different enzymes are involved. Postulation of a single α-galactosyl transferase would provide a simple explanation for the p phenotype, because individuals lacking this enzyme activity could not synthesize any of the three P-active glycolipids. This hypothesis would imply that individuals of the P_2 phenotype, who synthesize CTH and globoside but not the P_1 glycolipid, possess the α-galactosyl transferase required for synthesis of P_1 but lack the proper biosynthetic precursor. Analysis of the glycosyl-transferases and neutral glycolipids of the erythrocytes of p and P^k individuals will provide direct information about the nature of these abnormalities.

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