Review

An update on the GLOB blood group system and collection

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The P blood group antigen of the GLOB system is a glycolipid structure, also known as globoside, on the red blood cells (RBCs) of almost all individuals worldwide. The P antigen is intimately related to the P^k and NOR antigens discussed in the review about the P1PK blood group system. Naturally occurring anti-P is present in the serum of individuals with the rare globoside-deficient phenotypes p, P_1^k, and P_2^k and has been implicated in hemolytic transfusion reactions as well as unfavorable outcomes of pregnancy. The molecular genetic basis of globoside deficiency is absence of functional P synthase as a result of mutations at the *B3GALNT1* locus. Other related glycolipid structures, the LKE and PX2 antigens, remain in the GLOB blood group collection pending further evidence about the genes and gene products responsible for their synthesis. ***Immunohematology* 2013;29:19–24.**

History

Reading the early literature about what are currently known as the P1PK and GLOB blood group systems is a bit complicated because of evolving name changes based on increasing knowledge that has improved previously drawn conclusions. The P antigen is also known as globoside, a name given because it was discovered and characterized first on red blood cells (RBCs) (*globule rouge* is French for red blood cell). The antibody now referred to as anti-P1 was originally called anti-P and was initially recognized in 1955 as a component of anti-Tj^a (now designated anti-$PP1P^k$), the mix of naturally occurring antibodies in sera of people with the p phenotype.[1] The first globoside-deficient individual described had the rare P_1^k phenotype and was reported in 1959 by Matson et al.[2] However, the first paper highlighting the relationship between the P1, P^k, and P antigens, as well as determining the biochemical structure of these glycolipids, was published by Naiki et al.[3] 15 years later.

Terminology and Nomenclature

The P antigen is so far the only member of the GLOB blood group system acknowledged by the International Society of Blood Transfusion (ISBT) Working Party for Red Cell Immunogenetics and Blood Group Terminology as system number 028. The antigen was first assigned to the P blood group system (as antigen no. 003002), which is the former name of what is now known as the P1PK system (ISBT no. 003), today housing the P1, P^k, and NOR antigens. Thereafter, P was moved to the GLOB blood group collection (ISBT no. 209, antigen no. 209001) when it was clear that P1 and P were only distant relatives. Finally, it was promoted to form a blood group system of its own when the molecular genetic basis for P antigen synthesis was established in 2002 (antigen no. 028001).[4] Other names sometimes used instead of P, especially in the biochemical and glycobiological literature, include globoside, globotetraosylceramide, and Gb4. It should be noted that P was the name formerly used for what is now known as the P1 antigen. The LKE antigen remains in the GLOB collection (ISBT no. 209), together with the newly added PX2 antigen.[5] The current terminology of the GLOB blood group system and collection is summarized in Table 1.

Table 1. The GLOB blood group system and collection*

Antigen	ISBT system no.	ISBT collection no.	ISBT antigen no.
P	GLOB 028		028001
LKE		GLOB 209	209003
PX2		GLOB 209	209004

*209001 and 209002 are obsolete (previously used for P and P^k).

Molecular Genetic Basis of P Antigen

A gene, first cloned in 1998 as a member of the 3-β-galactosyltransferase family[6] but later shown to be a 3-β-*N*-acetylgalactosaminyltransferase, was suggested as the globoside (Gb4, P) synthase.[7] This gene (*B3GALNT1,* formerly known as *B3GALT3*) is located on the long arm of chromosome 3 (3q26.1) and has at least five exons with the entire coding region in the last exon (Fig. 1). The gene encodes the enzyme that synthesizes the P antigen. So far, 12 mutations have been found to abolish P synthase activity (Table 2).[4,8,9] Only one noncritical polymorphism has been described in the exons of this gene, in contrast to the P^k synthase gene (*A4GALT*), which varies more in the population.

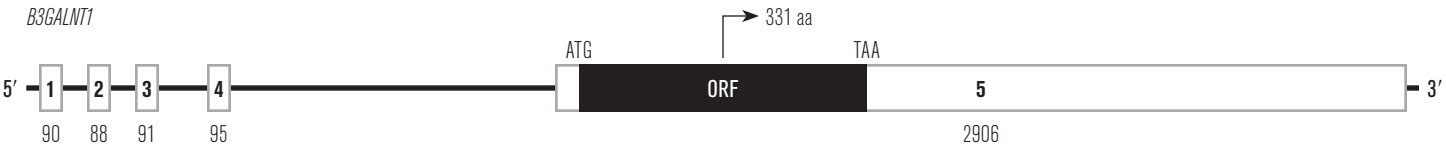

Fig. 1 The genomic organization of the P (*B3GALNT1*) gene, not drawn to scale. The numbers in the boxes represent the exon number and numbers below boxes represent the number of base pairs in the exon. The black box shows the open reading frame (ORF).

Table 2. A summary of all mutations found to date in the *B3GALNT1* gene[4,8,9]

	nt. position	202	203	292–293	433	449	456	537–538	598	648	797	811	959	
	Consensus	C	G	A	C	A	T	A	T	A	A	G	G	
ISBT allele name	Origin													Acc. no.
*GLOB*01N.01*	Finland	T												AF494103
*GLOB*01N.09*	Mahgreb		delG											FR871173
*GLOB*01N.02*	Italy			insA										AY505344
*GLOB*01N.03*	USA				T									AY505345
*GLOB*01N.12*	Turkish					G								FR871174
*GLOB*01N.11*	Saudi Arabian						G							FR871176
*GLOB*01N.04*	Arabic							insA						AF494104
*GLOB*01N.10*	French Caucasian								delT*					FR871175
*GLOB*01N.05*	Canada									C				AY505346
*GLOB*01N.06*	France										C			AF494106
*GLOB*01N.07*	Europe											A		AF494105
*GLOB*01N.08*	Switzerland												A	AY505347
	aa position	68	68	98	145	150	152	180	200	216	266	271	320	
	Consensus	R	R	R	R	D	Y	D	S	R	E	G	W	
	Change	stop	fs	fs	stop	G	stop	fs	fs	S	A	R	stop	

nt = nucleotide, Acc. no. = accession number, aa = amino acid, fs = frameshift.
*Additional mutation present in allele 376G>A (D126N).

Antigens and Antibodies in the System

The P antigen is present on RBCs of all individuals except ones with the rare phenotypes p, P_1^k, or P_2^k. The P_1^k phenotype lacks the P antigen on the cell surface, whereas the P_2^k phenotype lacks both the P and P1 antigens. The p phenotype, on the other hand, lacks P^k, P, and P1 antigens. Additional phenotypes might exist as Kundu et al. described individuals with either a weak P or weak P^k antigen,[10,11] but the genetic basis of this is not known and such individuals appear to be rare.

The P_1^k and P_2^k phenotypes are even rarer than the p phenotype but appear to be more common in Japan.[12] The first individual described with the P^k phenotype was of Finnish origin, and it also appears that Finland has a higher prevalence of the rare P_1^k and P_2^k phenotypes than do other populations.[13,14]

The antigen is well developed at birth[13] and is the most abundant neutral glycolipid in the RBC membrane with approximately 15×10^6 antigens per cell,[15] probably the highest antigen site density for any blood group antigen. None of the enzymes or chemicals used to treat test RBCs for antigen modification can abolish its expression, but many, including papain and trypsin, markedly enhance it. RBCs from P_1 individuals express more P^k antigen compared with P_2 individuals, but the amount of P antigen is similar for both phenotypes.[15] However, because the P^k antigen constitutes the precursor for P synthase, it is possible that the P antigen site density is somewhat lower on P_2 individuals, but substantial interindividual variation exists.

Naturally occurring antibodies of IgM or IgG classes are formed when the P antigen is missing. In analogy with ABO antibodies, anti-P can cause hemolytic transfusion reactions of the acute intravascular type, although no clinically significant hemolytic disease of the fetus and newborn has been reported. Nevertheless, early spontaneous abortions occur with a higher frequency among women with p and P_1^k or P_2^k phenotype; this is a phenomenon most likely attributable to the IgG component of anti-P attacking certain cells in the placenta, where globoside

is highly expressed.[16,17] Plasmapheresis to reduce the maternal antibody titer has been successfully used in selected cases.[18] Autoanti-P can be formed after viral infections (see Disease Associations).

Biochemistry

P antigen belongs to the globoseries of glycolipids. Its structure was first elucidated by Naiki et al., and it was later shown by Yang et al. that glycolipids are the sole carriers of P antigen on RBCs.[3,19] The P antigen is made with the addition of *N*-acetylgalactosamine (GalNAc) by a 3-β-*N*-acetylgalactosaminyltransferase (β3GalNAc-T1 classified as EC 2.4.1.79 but also known as P or Gb4 synthase) using P^k (Gb3) as a precursor (Fig. 2). This enzyme is a type II transmembrane glycoprotein with 331 amino acids and five potential *N*-glycosylation sites. Similar to other glycosyltransferases, it resides in the Golgi apparatus, where it is part of the glycosylation machinery of the cell. The crystal structure of this enzyme has not yet been solved, and regulation of its transcription is not well understood either.

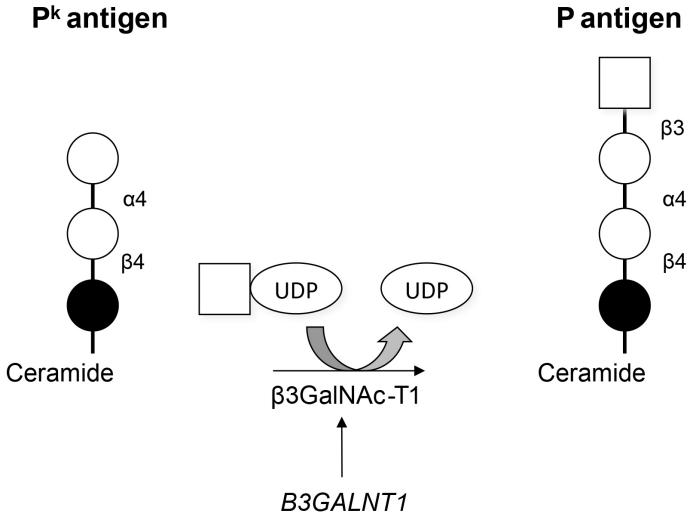

Fig. 2 Schematic summary of the synthesis of P antigen. The α- or β-linkages are shown along with the informative numbers in the 1,3 or 1,4 glycosidic bonds.
● Glucose (Glc) ○ Galactose (Gal)
□ *N*-acetylgalactosamine (GalNAc)

Tissue Distribution

Expression of the P antigen has been studied in several species.[20–22] Studies of mouse tissues show expression patterns similar to those of humans, although there are some differences.[21]

The P antigen is expressed on a number of other cells in addition to RBCs, but various studies using different antibodies or methods have come to different conclusions about where it is expressed. Soluble P substance has also been detected in plasma.[23,24] Small amounts of P^k, together with higher amounts of P, are present on intestinal epithelial cells.[25] P antigen expression is also seen on megakaryocytes and fibroblasts but not on lymphocytes and granulocytes according to von dem Borne et al.,[26] whereas Shevinsky et al.[27] could not detect P on fibroblasts. In another study, P was detected in 11 of 16 investigated tissues, especially those of mesodermal origin.[28] Placenta and fetal heart and kidney are other tissues where P is present.[17] The P antigen is expressed on embryonal carcinoma cells and, according to Song et al.,[29] is a possible initiator of signal transduction through AP-1 and CREB, associated with cell adhesion. High expression of the *B3GALNT1* gene has been demonstrated in brain and heart, moderate expression in lung, placenta, and testis, and low expression in kidney, liver, spleen, and stomach.[7]

Disease Associations

Parvovirus B19, which causes the so-called fifth disease, uses erythroid precursor cells expressing high levels of P antigen for its replication.[30,31] Infection during pregnancy with B19 can give rise to fetal anemia and, in some cases, fetal loss as a result of inhibition of hematopoiesis.[32] Paroxysmal cold hemoglobinuria, which can be seen in children after a viral infection, can be caused by an autoanti-P. This complement-fixing and cold-reactive antibody, also called Donath-Landsteiner antibody, lyses autologous P-positive erythrocytes.[33] P-fimbriated uropathogenic *Escherichia coli* that express *Pap*-encoded adhesins bind to glycolipid structures containing the Gal4Gal motif and can cause urinary tract infections such as cystitis and pyelonephritis. This includes the P antigen and other globoseries antigens like P^k, as well as the neolactoseries P1 antigen.[34,35]

Related Antigens in the GLOB Collection

The LKE (Luke) Antigen

The Luke (LKE, also known as SSEA-4 or monosialogalactosylgloboside, MSGG) antigen was found in 1965[36] and named LKE in 1986 after the first patient with anti-LKE. Since 1990, LKE belongs to the GLOB collection (ISBT no. 209). The antigen, which was given number 209003, is formed by sequential addition of a galactose (Gal) moiety and thereafter sialic acid (NeuAc) to the P antigen to form

galactosylgloboside (Gb5) and LKE, respectively. Although the enzyme responsible for Gb5 synthesis is thought to be encoded by *B3GALT5,* Gb5 is not known to elicit an antibody response and is therefore not classified as a blood group antigen. On the other hand, anti-LKE is found in LKE-negative individuals, but the enzyme responsible for synthesis of LKE has not yet been identified, which means LKE remains in the GLOB collection. Anti-LKE is a rare finding, and no clinically significant reactions have been reported. There are three different LKE phenotypes: strongly positive (80–90% of individuals), weakly positive (10–20%), and negative (1–2%). In addition to RBCs, LKE is expressed on endothelial cells, smooth muscle cells, kidney cells, platelets, and mesenchymal stem cells.[37–39] Individuals with the LKE-negative phenotype express more P^k antigen compared with individuals with LKE-positive phenotype.[40]

The PX2 Antigen

The PX2 antigen, a high-prevalence antigen originally designated as the x_2 glycolipid, was first mentioned by Kannagi et al.,[41] and the structure was later characterized further by Thorn et al.,[42] who also found it elevated on RBCs with the p phenotype. It is formed by addition of a β3GalNAc to paragloboside, the same precursor used by the FUT1 enzyme to make H antigen or P1 synthase to make P1. There are also sialylated variants of x_2 described.[42]

In individuals with the P_1^k phenotype only anti-P is expected in plasma according to textbooks, but it was recently reported that another naturally occurring antibody specificity could give rise to a weak or variable crossmatch reactivity with p phenotype RBCs owing to their elevated amounts of x_2 glycolipid.[5] At the same time, all P_1^k and P_2^k RBCs were compatible. In the absence of a functional β3GalNAc transferase because of *B3GALNT1* mutations in P_1^k and P_2^k phenotype individuals, it is hypothesized that paragloboside can no longer be extended to form PX2 but this has yet to be proved. In the meantime, the PX2 antigen has been assigned to the GLOB collection (ISBT no. 209).[43]

The biochemical and genetic relationship between the GLOB system and collection antigens discussed in this review is summarized in Figure 3.

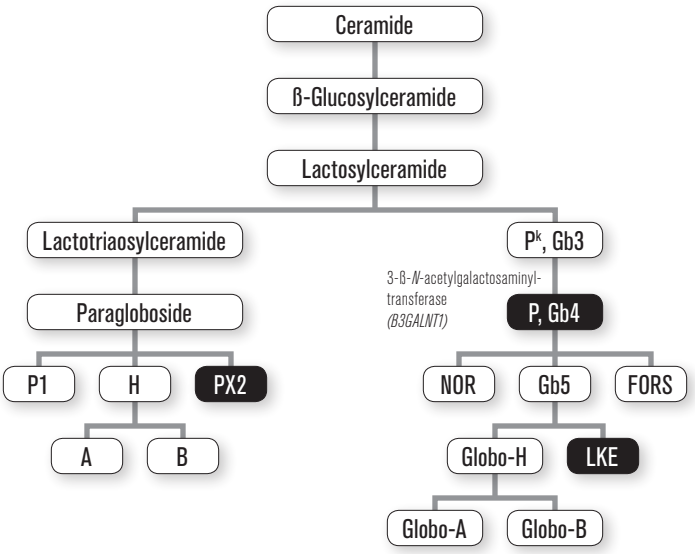

Fig. 3 The biochemical and genetic relationship between the antigens discussed in this review and other related carbohydrate antigens.

Summary

The GLOB blood group system currently consists of only one antigen, P, or globoside, but is closely associated with the antigens in the P1PK blood group system as well as the GLOB collection antigens. The P antigen can act as a receptor for various pathogens and anti-P can cause hemolytic transfusion reactions, spontaneous recurrent abortions, and autoimmune anemia as a result of lysis of erythroid progenitors.

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