P1PK: The blood group system that changed its name and expanded

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The antigens in the P1PK blood group system are carried on glycosphingolipids. The system currently includes three different antigens, P1, P^k, and NOR. The P1 antigen was disovered in 1927 by Landsteiner and Levine, and P^k and NOR were described in 1951 and 1982, respectively. As in the ABO system, naturally occurring antibodies of the immunoglobulin (Ig) M or IgG class, against the missing carbohydrate structures, can be present in the sera of people lacking the corresponding antigen. Anti-P1 is generally a weak and cold-reactive antibody not implicated in hemolytic transfusion reaction (HTR) or hemolytic disease of the fetus and newborn while P^k antibodies can cause HTR, and anti-NOR is regarded as a polyagglutinin. A higher frequency of miscarriage is seen in women with the rare phenotypes p, P_1^k, and P_2^k. Furthermore, the P^k and P1 antigens have wide tissue distributions and can act as host receptors for various pathogens and toxins. Why p individuals lack not only P^k and P expression but also P1 has been a longstanding enigma. Recently, it was shown that the same *A4GALT*-encoded galactosyltransferase synthesizes both the P1 and P^k antigens and that a polymorphism in a new exon in this gene predicts the P_1 and P_2 phenotypes. ***Immunohematology* 2013;29:25–33.**

Historical Aspects

In 1927, Landsteiner and Levine[1] found antibodies to an antigen they called P (now known as the P1 antigen). In 1951, the Jay blood group "system" was discovered, containing one antigen, Tj^a, and its corresponding antibody, anti-Tj^a.[2] Four years later, Sanger[3] found a relationship between the P (P1) and the Jay systems. The P (P1) antigen was absent from all red blood cells (RBCs) with the rare Tj(a–) phenotype. The antigen P was then renamed to P1 and the Tj(a–) phenotype became the p (or PP1P^knull) phenotype.[3] Anti-P was originally 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.[3] The first individual lacking the P antigen was described in 1959 by Matson et al.,[4] and in the same paper the P^k antigen and anti-P^k were first mentioned. The authors also noted the association with the P blood group system.[4]

Nevertheless, the relationship between the different antigens was not fully understood at that time. In the fifth edition of *Blood Groups in Man,*[5] the authors declare "we began to feel lost in amazement at the complexity of the P system." Indeed, this feeling has lingered (and still does) among many of us who work with these questions. However, the first paper explaining the relationship between the P1, P^k, and P antigens, as well as determining the biochemical structure of these glycolipids, was published in 1974 by Naiki et al.[6] See Figure 1 for a schematic representation of the antigens discussed in this review.

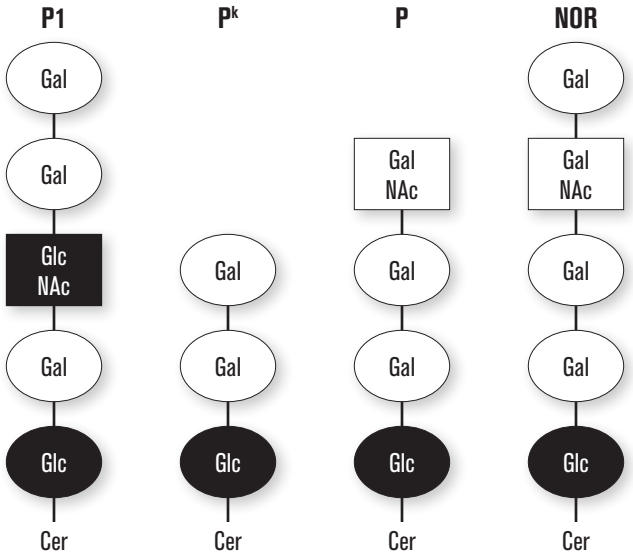

Fig. 1 The antigens in the P1PK blood group system, together with the related P antigen.

Nomenclature

The history of nomenclature for the P1 and P^k antigens is complicated and sometimes confusing. The P1 antigen, originally called P, used to belong to the P blood group system and the P^k antigen to the GLOB collection (ISBT no. 209). However, the P antigen (globoside, Gb4) did not belong to the original P system, but has now been made the only antigen in the GLOB blood group system (ISBT no. 028).[7,8] Now that it is clear that the *A4GALT* gene is responsible for both the P1 and P^k antigens (see later section), the P^k antigen has joined the P1 antigen and the system name has been changed to the P1PK blood group system (ISBT no. 003) to reflect the two

major antigens in it (Table 1).[9] The NOR antigen was assigned to the P1PK system by the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology at the 2012 annual meeting in Cancun (Table 1).[10] The related antigens LKE and PX2, as well as P, are discussed in the GLOB blood group system review also published in this issue.[11]

Table 1. The P1PK blood group system

Antigen	Blood group system	ISBT number
P1	P1PK	003001
P^k	P1PK	003003
NOR	P1PK	003004

003002 is obsolete.

The P^k antigen is also known as the Burkitt lymphoma antigen and has been classified as CD77.[12] Yet another name, Gb3, is often used when the P^k structure is expressed on cells other than RBCs. This is in analogy with Gb4 being an alternative and more biochemically useful name for P.

Biochemistry

The P1PK and GLOB antigens are related and are structurally of a glycan nature. Depending on which carbohydrate residues are added to lactosylceramide (LacCer), different series of glycosphingolipids are formed. Glycosphingolipids were first described by Thudichum in 1884, and he named them after the inscrutable Egyptian Sphinx, as both the structure and the function were unknown at the time.[13] These molecules consist of a sugar moiety with a lipid ceramide tail; they make up the outer leaflet of cell membranes together with phospholipids, cholesterol, and glycerolipids. Lipids are organised in microdomains such as glycosynapses and lipid rafts.[14]

The P1PK antigens, as well as the GLOB system and collection antigens, are formed on the same precursor, LacCer, which is the most common precursor for glycosphingolipids in mammals and birds.[15] P^k (other names include globotriaosylceramide, Gb3, ceramide trihexoside, and CTH) belongs to the globo-series, and P1 (also known as nLc5) to the neolacto/paraglobo-series.

The biochemistry of the P1 blood group antigen was partially elucidated by Morgan and Watkins[16] in the 1960s by a series of experiments on hydatid cyst fluid from sheep infected by the tapeworm *Echinococcus granulosus*. They purified P1-specific components and showed that a glycoprotein containing the Galα1-4Galβ1-4GlcNAc trisaccharide reacted as a P1 determinant. Some years later, Marcus[17] managed to extract P1 glycolipids from RBCs. In 1974, the P^k structure was identified as CTH by Naiki and Marcus.[6]

The 4-α-galactosyltransferase (α4Gal-T/$P1P^k$ synthase) catalyzes the transfer of galactose to the galactose residue on LacCer, producing the P^k antigen. In another pathway, the P1 antigen is formed by three sequential glycosylation reactions, the last one performed by the $P1P^k$ synthase. Furthermore, other glycosyltransferases form additional blood group antigens associated with the P1PK/GLOB systems and collection, such as Forssman (FORS1), globo-H, and globo-A (Fig. 2).[18–20]

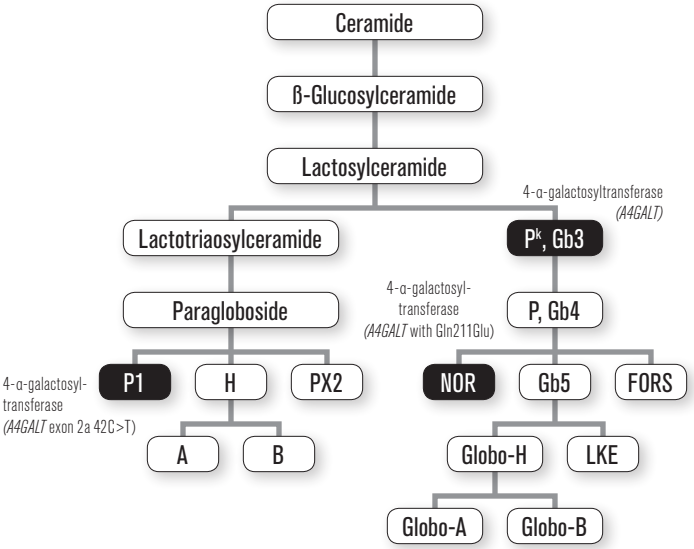

Fig. 2 The biochemical and genetic relationship between the antigens (black boxes) of the P1PK blood group system and other related carbohydrate antigens.

It has been debated whether the P^k and P1 antigens exist on glycoproteins in the human RBC membrane, but according to Yang et al.[21] glycolipids are the sole carriers of these antigens on RBCs.

Antigens and Antibodies in the System

Different combinations of the P1, P, and P^k antigens give rise to the following phenotypes: P_1, P_2, P_1^k, P_2^k, and p (Table 2). The prevalence of the P1 phenotype varies among different ethnic groups, ranging from 90 percent among Africans to 80 percent in whites down to 20 percent in Asians.[10,23] On RBCs, P1 expression changes during fetal development. The antigen is found as early as week 12 but weakens during gestation.[24] At birth the expression is low, and it takes up to 7 years before full expression is reached.[25] The strength of the antigen expression can differ from one person to another, and it was proposed

as early as 1953 to be dependent on dosage.[26] The discovery of a P^1/P^2-predictive single-nucleotide polymorphism (SNP) did indeed prove this proposal to be correct, and it could be confirmed with traditional serology, antigen site density measurement by flow cytometry, and quantification of transcription levels by real-time polymerase chain reaction.[27]

Table 2. A summary of phenotypes and possible antibodies for the P1PK/GLOB blood groups

Phenotype	Prevalence	Antigens present on RBC	Antibodies in serum
P_1	20–90%	P1, P^k, P	—
P_2	10–80%	P^k, P	Anti-P1*
p	rare	—	Anti-PP1P^k
P_1^k	rare	P1, P^k	Anti-P**
P_2^k	rare	P^k	Anti-P and P1**

*Not always present, detectable, or both.
**Anti-PX2 can be present.[22]

Individuals with the In(Lu) phenotype express lower amounts of P1 antigen.[28] In 2008 Singleton et al.[29] found that the majority (21 of 24) of such individuals are heterozygous for mutations in their *EKLF* gene. This suggests that expression of P^k and P1 on RBCs may depend on binding of the erythroid transcription factor EKLF to the *A4GALT* promoter.

The P^k antigen was first thought to be a low-prevalence antigen, but later it was understood that nearly all the antigens are masked by addition of β3GalNAc to form globoside, the P antigen.[30] RBCs from P_1 individuals express more P^k antigen compared with those from P_2 individuals.[27,31] The rare null phenotype, p, lacks the P^k, P, and P1 antigens (Table 2). However, additional phenotypes might exist: Kundu et al.[32,33] described individuals with either a weak P or a weak P^k antigen.

The frequency of the p phenotype has been estimated at 5.8 per million in Europeans,[34] but for Swedes in Västerbotten county in Northern Sweden the number of p individuals is significantly higher (141 per million).[35] The phenotype also seems to be more common in Japan and among Amish people.[36,37] The frequency of p in the donor population in Israel is comparable to that in other populations, but among Jews who immigrated to Israel from North Africa the p phenotype prevalence is 10 times as high.[38]

Naturally occurring antibodies of the IgM, IgG, or both classes are formed against the missing P1/P^k carbohydrate structures (Table 2), in the same way as they are against the A/B carbohydrate structures in the ABO blood group system.

Anti-P1 is usually a weak and cold-reactive antibody not implicated in hemolytic transfusion reaction (HTR) or hemolytic disease of the fetus and newborn (HDFN). However, some antibodies against P1 have been reported to react at 37˚C, bind complement, and cause both immediate and delayed HTRs.[39–41]

Anti-PP1P^k can cause HTR, but HDFN has not been reported. Instead, some of the women with anti-PP1P^k, or with anti-P in the globoside-deficient null phenotypes of the GLOB blood group system, suffer from recurrent spontaneous abortions.[42,43] The fetus as well as the newborn express low amounts of the P1, P, and P^k antigens, but the placenta shows high expression and is therefore a possible target of the antibodies that may be the cause of the miscarriages.[43] It has been suggested that it is mainly the IgG anti-P component in women with the p phenotype that attacks the fetally derived cells in the placenta.[42,43] In a group of 17 female Swedish p individuals, 11 had experienced at least one spontaneous abortion.[44]

The anti-PP1P^k found in individuals with the p phenotype was previously called anti-Tj^a, named after Mrs. J in whose serum this antibody specificity was first found in association with a tumor.[2]

NOR antigen expression, a low-prevalence polyagglutinable state, was first described in 1982 in a patient living in Norton, Virginia, hence the name.[45] So far, it has only been found in two families, one in the United States and one in Poland.[45,46] The RBCs of the initial patient were agglutinated by 75 percent of tested ABO-compatible sera but were not agglutinated by any lectin known to react with other types of polyagglutinable RBCs. The polyagglutination was inhibited by avian P1 substance, and a possible relation to the P1PK blood group system was suggested.[45] Family studies showed that the antigen was inherited and that anti-NOR is present in most adult human sera; it has therefore been called a polyagglutinin. It is unknown whether this kind of antibody is clinically significant, because transfusion of NOR+ blood is such a rare event.[10]

Genetics and Molecular Basis

The *P1PK* (*A4GALT*) Gene

The gene encoding the implicated 4-α-galactosyltransferase (α4Gal-T, EC 2.4.1.228) was cloned in 2000 by three independent research groups[47–49] and was originally only found to give rise to the P^k antigen. The gene is located on the long arm of chromosome 22 and consists of four exons with the whole coding region in the last exon (Fig. 3). This gene encodes a type II transmembrane glycoprotein with 353 amino acids and is highly conserved among different species.[48,50] The protein sequence contains a characteristic DXD motif (amino acids 192–194), which is a conserved motif existing in nearly all glycosyltransferases.[51] It has been

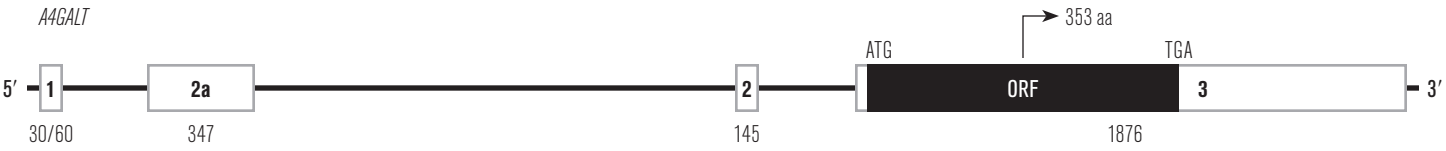

Fig. 3 The genomic organization of the *A4GALT* gene (not drawn to scale). The numbers in the boxes represent the exon number and numbers below the boxes represent the number of base pairs in the exon. The black box shows the open reading frame (ORF).

proposed that this motif participates in the coordination of the metal ion and therefore also in the binding of the nucleotide part of the uridine diphosphate donor sugar.[51] The crystal x-ray structure of the enzyme has not been solved yet, and the type of cation *A4GALT* required for its function has not been clarified. The promoter region of the gene has binding sequences for the transcription factor AP-1 (TGAGTCA), 160 bp upstream of the transcription start according to a computer search done by Hughes et al.[52] In another study, it was shown that the promoter contains three binding sites for Sp1, four GC boxes, but no TATA or CCAAT boxes.[53] However, a specific reason why this gene is expressed in erythroid tissues has not been reported.

A major enigma has been why the P1 antigen is always absent in the p phenotype. Different theories have been proposed. One model suggested that the same enzyme, α4Gal-T, is able to transfer galactosyl residues to both LacCer and paragloboside, but to use the latter as the acceptor, a regulatory protein is required.[54] Another hypothesis suggested that two different enzymes exist, both of which must be inactivated to cause the p phenotype.[54] This model was supposedly supported by a study showing that microsomal enzymes from P_1 kidneys could synthesize both P_1 and P^k, whereas enzymes from P_2 kidneys could only produce P^k.[55] A third model proposed a single gene with three alleles, one allele coding for a α4Gal-T using LacCer and paragloboside as the possible acceptors, one allele using LacCer only, and the third allele coding for an inactive form of the transferase.[56] However, no polymorphisms in the coding region of the *A4GALT* gene appeared to explain the P_1/P_2 phenotypes. Hence the theory with one gene encoding for both P1 and P^k was temporarily abandoned.[47] Iwamura et al.[57] proposed that transcriptional regulation, caused by two different polymorphisms in the 5′-regulatory region of *A4GALT,* might instead be the underlying reason for the P_1/P_2 phenotypes. However, these findings could not be verified in their own transcription assay or in two other independent studies.[58,59]

In 2010, it was demonstrated that the *A4GALT* product could also synthesize P1 antigen.[60] A genetic marker with which prediction of P_1 versus P_2 phenotype could be achieved was reported when a novel *A4GALT* transcript, containing exon 1 and a new exon, designated exon 2a, was discovered.[27] This exon contains a P_1- versus P_2-associated polymorphism (42C/T), which also opens a short potential reading frame in P^2 alleles. However, the mechanism by which this SNP operates is still unknown, even if 42T was shown to be correlated to lower *A4GALT*-mRNA levels.[27] The authors hypothesize that either the new transcript, the hypothetical P^2-related peptide, or the SNP in the genomic sequence itself may downregulate the transcription of *A4GALT* in P_2 individuals. Alternatively, yet other polymorphisms closely linked in *cis* with this SNP could be involved. Several candidate SNPs occur in close proximity to exon 2a and 42C/T, as do potential binding sites for erythroid transcription factors. No matter which mechanism is active, typing for the 42C/T polymorphism correctly predicted the P_1/P_2 phenotype in 207 of 208 common Swedish blood donors, and full concordance was recently obtained also in 200 Asian donors (100 P_1 and 100 P_2).[27,61]

To date, 29 mutations in 33 alleles of the *A4GALT* gene have been found to cause the p phenotype.[44,62,63] For a complete list of these mutations and alleles, see the homepage of the International Society of Blood Transfusion (ISBT; www.isbtweb.org). Two silent polymorphisms, 903G>C (P301P) and 987G>A (T329T), as well as one missense mutation, 109A>G (M37V), with no apparent effect on the α4Gal-T, have also been documented.[47] These polymorphisms were found to be organized into different haplotypes and linked to upstream and downstream SNPs.[58] In addition, a few other noncritical polymorphisms have been found in combination with enzyme-crippling, p-associated mutations.

A missense mutation in the *A4GALT* gene causing an amino acid change, Gln211Glu, was found in individuals positive for the rare NOR antigen.[64] This is believed to make the *A4GALT*-encoded enzyme add a galactose to the P antigen, thereby forming NOR. Thus, in addition to making P1 and P^k antigen, the Gln211Glu form of α4Gal-T also makes NOR. We sequenced the *A4GALT* genes of NOR+ family members and found that the NOR-specific SNP (631C>G) was linked to the P1-associated exon 2a SNP 42C.[64] Further elongation of the NOR antigen (also called NOR1) can give rise to NORint and NOR2,[65] two glycolipid structures not yet given blood group status by the ISBT.

Tissue Distribution

The expression of glycosphingolipids often shows a wide histological distribution but varies among tissues and species. Expression of the *A4GALT*-derived P^k and P1 antigens has been studied in several species.[48,66,67] Studies on mouse tissues show expression patterns similar to those found in humans, although some differences have been noted.[66]

The P1 structure is found as glycolipids, glycoproteins, or both in many organisms such as the nematode *(Ascaris suum),* tapeworm *(Echinococcus granulosus),* earthworm *(Lumbricus terrestris),* liver fluke *(Fasciola hepatica),* bacterium *(Neisseira gonorrhoe),* and pigeon.[68] The P^k antigen is also expressed in several strains of bacteria.[69]

In humans, glycosphingolipids can be useful as surface markers of normal erythrocyte differentiation and of erythroleukemias.[70] The P^k and P1 antigens are 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 they are expressed. P^k has been detected in plasma,[41,71] but no such reports about P1 in plasma or about P^k and P1 in secretions are available.

The P1 antigen is expressed on B lymphocytes, granulocytes, and monocytes.[72] The P^k antigen has been found on all leucocytes (except NK cells),[72] fibroblasts,[30] platelets, and smooth muscle cells of the digestive tract and urogenital system[73] and is a differentiation antigen expressed on a subset of tonsillar B cells in the germinal center.[74] High expression of P^k in the kidney has been implicated in susceptibility to hemolytic uremic syndrome (HUS), further discussed in the section on disease associations. The mechanism behind the high renal expression might be related to enhanced *A4GALT* gene transcription and reduced α-galactosidase gene activity.[52] Recently, it has also been shown that small amounts of P^k together with higher amounts of P are present on intestinal epithelial cells.[75]

Northern blot studies of human organs showed high expression of the *A4GALT* gene in kidney and heart in one study,[47] while another report described high expression in spleen, liver, testis, and placenta, in addition to kidney and heart.[49]

Disease Associations

The P^k and P1 antigens can act as membrane receptors for several pathogens and toxins, summarized in Table 3.

Table 3. A selection of pathogens and toxins with a relationship to the P^k and P1 antigens

Pathogen/toxin	Disease	Antigen involved	Reference
HIV	AIDS	P^k	76,77
Uropathogenic *Escherichia coli*	UTI	P^k, P1	78,79
Streptococcus suis	Meningitis	P^k, P1	80
Shigella dysenteriae (Shiga toxin)	Dysentery	P^k	81
Escherichia coli O157 (Stx 1/2)	HUS, hemorrhagic colitis	P^k	81,82
Pseudomonas aeruginosa (PA-IL lectin)	Opportunistic human pathogen	P^k, P1	83

HIV = human immunodeficiency virus; AIDS = acquired immune deficiency syndrome; UTI = urinary tract infection; HUS = hemolytic uremic syndrome.

Viruses

Although initial studies implied a facilitating role for P^k in human immunodeficiency virus (HIV) infection, recent work has suggested that P^k is protective when accumulated owing to reduced activity of α-galactosidase A in Fabry disease, an X-linked lysosomal storage disorder.[84] In addition, a soluble analogue of P^k prevents HIV infection in vitro.[77,85] Another study showed that peripheral blood mononuclear cells (PBMCs) with P_1^k phenotype were highly resistant to infection, whereas PBMCs with the p phenotype showed increased susceptibility to infection.[76] Furthermore, P^k-liposome fusion into the P^k-deficient Jurkat T-cell line reduced productive X4 HIV-1 infection, as did overexpression of P^k synthase. Accordingly, siRNA silencing of the P^k synthase gene increased the cells' HIV susceptibility.[76]

Bacteria

Uropathogenic *Escherichia coli* expressing *pap*-encoded PapG adhesins bind to P^k and P1,[78,79] and both the *Streptococcus suis* adhesin and the PA-IL lectin from *Pseudomonas aeruginosa* use P^k (Gb3) and P1 as receptors.[79,80,83,86] Furthermore, P^k is the receptor for Shiga toxin from *Shigella dysenteriae* (Stx) or certain *E. coli* strains (Stx1 and Stx2) on renal epithelium, platelets, and endothelium.[81,82]

A disease connected to the P^k antigen is Fabry disease, in which deficiency of the lysosomal enzyme α-galactosidase A causes accumulation of sphingolipids, mainly P^k, in some cell types and body fluids.[87] It has been shown that mice with Fabry disease are protected against Stx from enterohemorrhagic *E. coli* (EHEC).[88] These data are surprising because P^k is the cellular receptor for Stx and therefore a higher sensitivity would be expected. The authors hypothesize that the excess P^k can work as a toxin sink, which allows the toxin to bind to P^k in tissues that normally do not have high expression

and cannot be affected by the toxin. EHEC infection can induce HUS, which leads to hemolytic anemia, renal failure, and thrombocytopenia.[89] According to Furukawa et al.,[90] the mechanism behind the thrombocytopenia might be that Stx binds to P^k in immature megakaryoblasts and induces their apoptosis, leading to the restraint of platelet production in the bone marrow. The P^k antigen has been shown to mediate apoptotic signals after the binding of both Stx and anti-P^k (CD77 monoclonal antibody). These ligands trigger two completely different apoptotic pathways, one caspase- and mitochondria-dependent and one reactive oxygen species–dependent.[91] It has also been shown that patients with HUS have lower levels of P^k glycolipid in their sera compared with a healthy control group.[92] These authors propose that circulating Stx could bind to P^k glycolipids in sera during infection, which may reduce the amount of Stx binding to the target cells. Consequently, patients with low serum levels of P^k would have a higher susceptibility to EHEC infections. Another study states that only P^k and not P1, as earlier believed, is the receptor for Stx, and mice without P^k lose sensitivity to Stx.[93]

Cancer

Altered glycosylation patterns of glycosphingolipids such as neoexpression, underexpression, or overexpression are characteristic of cancer cells.[94] One example is the first described p individual (lacking P^k, P, and P1 antigens), who had a gastric tumor that expressed P1 antigen. Levine[95] proposed that the antibodies made against the P^k, P, and P1 antigens prevented further growth of the tumor. Altered expression of P^k antigen has also been described in ovarian carcinomas, colon cancer, breast cancer, and B-cell lymphomas.[96] It has even been suggested that Stx, which specifically binds to P^k, could be used as a targeted cancer therapy.[96]

Summary

The history of the P1PK blood group system is complex and not easy to grasp because of several changes of nomenclature. In addition, the biochemical background and genetic basis have caused long debates, and even today many basic questions remain to be solved. However, step by step the biochemical and genetic basis underlying the antigens expressed in this system has been revealed. The most recent but certainly not the final step came when it was clarified that the *A4GALT* gene is responsible not only for P^k expression but also for P1 and even NOR expression. As a result, the P^k and later the NOR antigen joined the P1 antigen, and the system name was changed to P1PK.

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