4 P1PK, Globoside, and FORS Blood Group Systems, plus Some Other Related Blood Groups

4.1 Introduction

The antigens described in this chapter are classified into three blood group systems and a collection (Table 4.1).

Whilst looking for new polymorphisms by injecting rabbits with human red cells, Landsteiner and Levine [1] discovered the P (now P1PK) blood group system in a series of experiments that also revealed the MN groups. After removing anti-species agglutinins, the immune sera were tested for antibodies that reacted differently with red cells from different people. One such antibody, which could not be explained by ABO or MN, defined two types of blood, now called P1+ (or P_1 phenotype) and P1– (or P_2 phenotype). Human alloantibodies of the same specificity were soon found.

The P system was expanded in 1955 by Sanger [2], who observed that red cells of the very rare phenotype Tj(a–) were always P1– and Tj(a–) was renamed p. Recognition in 1959 of another rare phenotype, P^k, created further complexity [3]. P^k red cells have strong expression of P^k antigen and lack a high frequency antigen, now called P, which is strongly expressed on all other red cells except those of the p phenotype (Table 4.2). P^k red cells may be P_1 or P_2. The Luke antigen (LKE) is another associated antigen of relatively high incidence lacking from p cells [4]. The reactions of antibodies defining these phenotypes are shown in Table 4.2.

The first biochemical steps were taken by Morgan and Watkins [5], who isolated a P1-active glycoprotein from hydatid cyst fluid (HCF). The P1 determinant was identified as a trisaccharide [6]. The identification of the P1, P, and P^k red cell antigens as glycosphingolipids (GSL) followed the work of Naiki and Marcus [7] in identifying the P antigen as the most abundant red cell glycosphingolipid, globoside (Gb4).

A single transferase encoded by a single gene (*A4GALT*) catalyses synthesis of P1 and P^k from different substrates [8]. Consequently, P1 (P1PK1) and P^k (P1PK3) belong to the P1PK blood group system. The product of another gene (*B3GALNT1*) catalyses synthesis of P from P^k, so P (GLOB1) is the only antigen of the globoside system. LKE (209002) and PX2 (209003) are classified in the 209 collection because their genetic backgrounds remain unclear. The rare FORS1 antigen, the Forssman glycolipid, is biochemically related to P [9].

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Table 4.1 Numerical notation for PIPK, globoside, and FORS systems, and for collection 209.

P1PK (System 3)		Globoside (System 28)		FORS (System 31)	Collection 209	
P1PK1	P1	GLOB1	P	FORS1	209003	LKE
P1PK3	P^k				209004	PX2
P1PK4*	NOR					

Obsolete: P1PK2, previously P; 209001, previously P; 209002, previously P^k.
*Provisional assignment.

Table 4.2 The P blood groups: phenotypes and antibodies.

Phenotype	Frequency in white people	Anti-P1	Anti-P	Anti-P^k	Anti-LKE	Anti-PP1P^k
P_1	75%	+	+	–*	+	+
P_2	25%	–	+	–*	+	+
p	Very rare	–	–/w	–	–	–
P_1^k	Very rare	+	–	+	–	+
P_2^k	Very rare	–	–	+	–	+
LKE+	98%	+ or –	+	–	+	+
LKE–	2%	+ or –	+	+†	–	+
Source of alloantibodies		P_2 people	P^k people	Anti-PP1P^k adsorbed with P_1 cells	LKE– people	p people

*Very weak P^k on these cells cannot be detected by agglutination tests with anti-P^k separated from anti-PP1P^k by adsorption with P_1 cells.
†P^k expression on LKE– cells less strong than P^k expression on P_1^k and P_2^k cells.
w, weak positive reaction.

4.2 Biochemistry, biosynthesis, and genetics

P antigenic determinants on red cells reside in the carbohydrate residues of glycosphingolipids, oligosaccharide chains attached to ceramide that form an important part of lipid raft microdomains [10]. Biosynthesis of the P antigens, like the ABH antigens, occurs by the sequential addition of monosaccharides to a precursor substrate, catalysed by glycosyltransferases. Two biosynthetic pathways are involved in production of these antigens, the globoside series and the paragloboside series, with a common precursor lactosylceramide (Gb2) (Table 4.3 and Figure 4.1). Reviews on P biochemistry include [11,12].

The early biochemical studies showed a close relationship between P1 and P^k, but gave no clue to the structure of P antigen. Using purified glycolipids to inhibit anti-PP1P^k, Naiki and Marcus [7] made the observation that globoside and ceramide trihexoside (Gb3), two very well-characterised glycolipids, constituted red cell P and P^k antigens, respectively. Characterisation of these antigens demonstrated that P^k was the direct precursor of P. Paragloboside (lacto-*N*-neotetraosylceramide) is a precursor of Type 2 ABH antigens, of some gangliosides, and of P1.

4.3 P1 (P1PK1) and anti-P1

4.3.1 Frequency and inheritance

The frequency of P1 varies in different populations. About 80% of white people are P_1. The frequency of P1 is much higher in some African and South American

Table 4.3 Structures of some glycosphingolipids associated with P antigens. P1 trisaccharide shown in red; NOR-active trisaccharide shown in blue.

Antigen	Structure	
	Lactosylceramide (Gb2)	Galβ1→4Glc-Cer
	Paragloboside series	
	Paragloboside	Galβ1→4GlcNAcβ1→3Galβ1→4Glc-Cer
P1	Galactosylparagloboside	Galα1→4Galβ1→4GlcNAcβ1→3Galβ1→4Glc-Cer
	Sialosylparagloboside	NeuAcα2→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc-Cer
PX2		GalNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc-Cer
	Globoside series	
P^k	Globotriosylceramide, Gb3	Galα1→4Galβ1→4Glc-Cer
P	Globoside (globotetraosylceramide) Gb4	GalNAcβ1→3Galα1→4Galβ1→4Glc-Cer
	Galactosylgloboside, Gb5	Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glc-Cer
LKE, SSEA-4	Sialosylgalactosylgloboside, MSGb5	NeuAcα2→3Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glc-Cer
	Disialosylgalactosylgloboside, DSGb5	NeuAcα2→3Galβ1→3(NeuAcα2→6)GalNAcβ1→3Galα1→4Galβ1→4Glc-Cer
H	Globo-H (Type 4 H)	Fucα1→2Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glc-Cer
FORS1	Forssman (Gb5)	GalNAcα1→3GalNAcβ1→3Galα1→4Galβ1→4Glc-Cer
NOR	NOR1	Galα1→4GalNAcβ1→3Galα1→4Galβ1→4Glc–Cer
	NOR_{int}	GalNAcβ1→3Galα1→4GalNAcβ1→3Galα1→4Galβ1→4Glc–Cer
NOR	NOR2	Galα1→4GalNAcβ1→3Galα1→4GalNAcβ1→3Galα1→4Galβ1→4Glc–Cer

peoples and very much lower in some Asian populations, as low as 30% in Japanese. For details of frequencies for many populations see [13]. In a survey of 2345 Scandinavians, 78.85% were P_1, providing the following gene and genotype frequencies:

P^1	0.5401	P^1/P^1	0.2917
P^2	0.4599	P^1/P^2	0.4968
		P^2/P^2	0.2115

Landsteiner and Levine [1] showed that P1 was inherited and behaved as a Mendelian dominant character. This is supported by all subsequent work.

4.3.2 Variation in strength

The strength of P1 on red cells shows individual variation and appears to be under genetic control [14–16]. Dosage contributes to this variation in strength, as confirmed by molecular genetic testing (Section 4.3.5). Fisher [17] analysed Henningsen's data and calculated that 66% of individuals with strong P1 were homozygous P^1/P^1 and all individuals with weak P1 were heterozygous P^1/P^2.

In(Lu), the rare dominant inhibitor of Lutheran and other red cell antigens that represents *EKLF* mutations, inhibits P1 expression [18,19] and has been responsible for P_2 parents with a P_1 child [19,20] (see Section 6.8).

4.3.3 Development and distribution

P1 is considerably weaker in children than in adults and the frequency of P_2 is substantially higher in newborn babies than in adults [14]. Complete development of P1 is not reached until seven years of age or older [21]. Despite this weak expression at birth, P1 is strongly expressed on fetal red cells. Fetal P1 expression is weaker than adult P1, but the strength of P1 decreases with increasing age of the fetus; P1 was more strongly, and more frequently, expressed by 12 week fetuses than by 28 week fetuses [22].

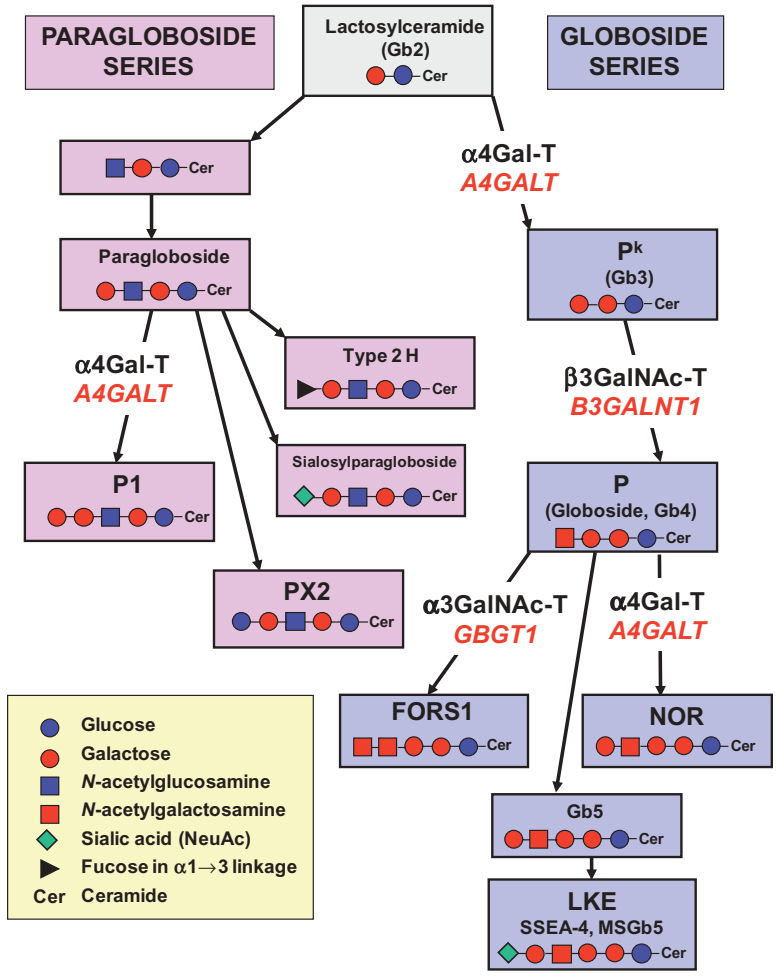

Figure 4.1 Biosynthetic pathways for formation of P and related antigens from a common precursor, lactosylceramide. Glycosyltransferases responsible for production of P1, P^k, P, and Forssman antigens and the genes that encode them are shown.

Flow cytometry with alloanti-P1 revealed that P1 is expressed on lymphocytes, granulocytes, and monocytes [23].

4.3.4 Other sources of P1 substance

Helminths (tapeworms and flukes) are sources of P1-active substances. Fluid from hydatid cysts of sheep livers inhibits anti-P1, but only if the fluid contains scolices [24]. The frequency and avidity of anti-P1 is increased in P_2 patients infested with certain helminths [24–27]. Annelid and nematode worms are also sources of P1 substance; extracts of *Lumbricus terrestris* (earthworm) and *Ascaris suum* inhibit anti-P1 [28].

Some other sources of P1 substance are avian in origin. Red cells, plasma, and excrement of pigeons and turtle doves, and ovomucoid of turtle dove egg white, all contain P1 substance [29–31]. Anti-P1 is more commonly found in P_2 pigeon-fanciers (34%) than in P_2 donors (6%) [29]. Substances like turtle dove ovomucoid and the hydatid cyst wall and protoscolices of helminths, which inhibit anti-P1 and can be used to stimulate anti-P1 production, have branching structures with the P1-trisaccharide (Table 4.3) [32,33].

4.3.5 Biochemistry and biosynthesis

The first information on the biochemical nature of P1 was derived from agglutination-inhibition tests, which

indicated the involvement of α-D-galactose in P1 specificity [34]. Morgan and Watkins [5] applied techniques previously used for extracting A, B, and H substances from body fluids to isolate a P1-active glycoprotein from HCF of sheep, which inhibits anti-P1. The products of partial acid hydrolysis of this glycoprotein led to characterisation of a trisaccharide, Galα1→4Galβ1→4GlcNAc as the P1 determinant [6].

P1 on red cells is a GSL [35–37]. After extensive purification, the structure of the active GSL was identified as the ceramide pentasaccharide shown in Table 4.3 [38,39], with the terminal trisaccharide identical to that isolated from the hydatid fluid P1 glycoprotein [6]. This structure is paragloboside with an additional non-reducing α-galactosyl residue.

P1-trisaccharide is very efficient at inhibiting monoclonal anti-P1 [40,41]. Synthetic glycoproteins containing the P1-trisaccharide have been used to immunise mice in the production of monoclonal anti-P1 [42].

The structure of P1 suggested that an α1,4-galactosyltransferase is responsible for the synthesis of P1 from paragloboside (Figure 4.1). The possibility that P_2 might reflect the lack of the precursor of P1 was eliminated by the observation that normal amounts of paragloboside were found in P_2 cells [43,44]. Red cells of the p phenotype lack P^k (Gb3), P (globoside), and P1 (Table 4.2). The p phenotype is associated with homozygosity for inactivating mutations in the P^k synthetase gene, *A4GALT*, explaining the absence of P^k (Section 4.10.2), but not the absence of P1.

Although P^k synthetase (α4Gal-T1) did not catalyse the synthesis of P1 from paragloboside *in vitro* [45] and no polymorphism correlating to P_1/P_2 phenotypes was detected in the coding region of *A4GALT* [45,46], incomplete associations between polymorphisms in the promoter region of *A4GALT* and the P_1/P_2 polymorphism suggested that the product of either *A4GALT* or of a closely linked gene was responsible for P1 synthesis [47–49]. In 2011, Thuresson *et al.* [8] confirmed that *A4GALT* is responsible for the P_1/P_2 polymorphism. *A4GALT* mRNA analysis revealed novel transcripts containing only the non-coding exon 1 and a 289/290-bp sequence (exon 2a) from intron 1. These transcripts contained three polymorphisms, one of which, 42C>T (counting from the first residue in exon 2a) was completely associated with P_1/P_2 phenotype. All P_2 samples were 42T/T, whereas P_1 samples were 42C/C or 42C/T. Nucleotide 42T introduces a putative start codon in P^2 alleles, potentially opening a short reading frame encoding 28 amino acids. *A4GALT* transcript levels were about 30 times higher in P_1 samples relative to P_2 samples. Comparison of P_1/P_2 phenotype with genotype confirmed that zygosity provided at least a partial explanation for variability in P1 antigen strength between P_1 individuals.

It is feasible to speculate that a genomic sequence, transcript, or peptide derived from the P^2 allele downregulates transcription at the *A4GALT* locus so that less enzyme is produced. As lactosylceramide is the favoured substrate, P^k is still synthesised at the expense of P1 synthesis from paragloboside, resulting in P_2 phenotype [8].

Identification of a SNP in exon 2a of *A4GALT* associated with the P_1/P_2 polymorphism makes it possible to predict P1 phenotype from genomic DNA [8].

4.3.6 Anti-P1

4.3.6.1 Alloanti-P1

Alloanti-P1 is a common specificity, usually a weak agglutinin active only at low temperature. Rarely has anti-P1 been attributed to stimulation by transfusion of red cells [50–53].

Most examples of anti-P1 do not agglutinate red cells at 25°C or above and these cold-reactive antibodies should not be considered clinically significant. There are two reports of immediate HTRs caused by anti-P1 that agglutinate red cells at 37°C; one had a fatal outcome [54,55]. Some examples have been reported to have caused delayed HTRs, although no anti-P1 was detected in the pretransfusion sample and, in one case, the antibody had disappeared within four months of the reaction [52,56]. Anti-P1 active at 37°C rapidly eliminated 50% of injected radiolabelled P1 cells; the rest were eliminated slowly [57]. Anti-P1 responsible for an immediate HTR gave a strongly positive result in an indirect monocyte monolayer assay with P1 red cells [55]. Patients with anti-P1 should be transfused with red cells compatible by IAT at 37°C. Anti-P1 has not been implicated in HDFN.

Anti-P1 has been found as a separable specificity in the serum of some p people by adsorption with P_2 cells, but anti-P1 has not been reported in any P_2^k individual. Alloanti-P1 in a P_1 pigeon breeder led to the suggestion that the antibody might be directed at a determinant absent from the patient's own P1 antigen [58].

4.3.6.2 Animal anti-P1

The first anti-P1 resulted from immunisation of rabbits with human red cells [1]. Since then, anti-P1 has been found as a 'naturally-occurring' antibody in rabbits and

other animals. Anti-P1 reagents have been made by injecting rabbits or goats with tanned P_2 cells that had been exposed to HCF [59], with partially purified P1 substance from sheep HCF coupled with a protein from *Shigella shigae* [34], with extracts of earthworms [28], or with soluble ovomucoid from turtle dove eggs [31].

4.3.6.3 Monoclonal anti-P1

Monoclonal antibodies with P1 specificity have been produced by immunising mice with turtle dove ovomucoid [40], with synthetic glycoproteins containing the P1-trisaccharide (Galα1→4Galβ1→4GlcNAc) [42], or with human red cells expressing strong P1 [60]. Agglutination of P1 red cells by monoclonal anti-P1 was inhibited by the P1 trisaccharide and by the disaccharide (Galα1→4Gal), the former being 200 times more efficient than the latter [40,41]. P1 monoclonal antibodies produced by immunisation with P1-trisaccharide bound equally well to the P1-trisaccharide and the P^k-trisaccharide (Galα1→4Galβ1→4Glc) [42].

4.4 P^k phenotype, P^k (P1PK3) antigen, and anti-P^k

4.4.1 P^k phenotype

Red cells of most people express P^k very weakly and P strongly, the P^k phenotype refers to those red cells that express P^k strongly and lack P. The expression of P^k on red cells of P^k people is uniformly strong regardless of P_1 or P_2 status; the variation in strength of P1 antigen is similar to that of P+ people. All P^k individuals have 'naturally occurring' anti-P in their serum, which reacts equally strongly with P_1 and P_2 cells. Most sera from P^k people react weakly with p cells, probably as a result of an additional antibody to the PX2 antigen [61] (Section 4.9).

All P^k propositi have been ascertained through anti-P in their sera. No random P^k individual has been reported despite the testing of 28 677 Finnish and 39 939 English donors [62]. P^k appears less uncommon in Finland and Japan than in other populations.

The red cells of parents of P^k propositi are not agglutinated by anti-P^k separated from anti-PP1P^k by adsorption with P1 cells, suggestive of recessive inheritance for the P^k phenotype, and a recessive mode of inheritance was supported by family studies [62–64]. P^k phenotype has a recessive mode of inheritance because it is the precursor of P antigen and is only detected by conventional serological methods on red cells of individuals homozygous for the gene responsible for inactive P synthetase (Section 4.4.2 and Figure 4.1).

4.4.2 P^k antigen, biochemistry, and biosynthesis

Initially red cells of people other than those with the rare P^k phenotype were thought to lack P^k antigen. Red cells of parents and children of P^k propositi were not agglutinated by anti-P^k (separated from anti-P1PP^k by adsorption with P_1 cells) and adsorption tests appeared to confirm this lack of P^k [65]. However, P^k was present on the fibroblasts of P_1 and P_2 individuals, and only absent from those of p people [66]. The following findings demonstrated that P^k is present on P+ red cells, though weakly expressed: the glycolipid Gb3 isolated from membranes of red cells of common phenotype inhibited anti-P^k [7], anti-P1P^k made by addition of globoside to anti-P1PP^k (to inhibit anti-P) agglutinated P_2 cells [67], and a monoclonal anti-P^k of high titre reacted weakly with P_1 and P_2 cells.

Red cells of P_1 LKE– and P_2 LKE– people have stronger expression of P^k antigen than those of individuals with the common P_1 LKE+ and P_2 LKE+ phenotypes, but weaker P^k expression than cells of $P_1{}^k$ and $P_2{}^k$ phenotypes, which are always LKE– [68,69] (Section 4.8).

The involvement of α-D-galactose in P^k specificity, first postulated by Voak *et al.* [70], was subsequently confirmed [71,72]. Anti-P^k, like anti-P1, is inhibited by HCF [3]. Partial acid hydrolysis of the P1P^k glycoprotein, isolated from HCF, yielded the P1-trisaccharide, which inhibited anti-P^k and -P1, and a disaccharide Galα1→4Gal, which inhibited anti-P^k, but not anti-P1 [72]. Other α-galactosyl-terminal oligosaccharides also inhibited anti-P^k [72], confirming the immunodominance of α-galactose in P^k expression.

P^k antigen is Gb3, which has the expected terminal Gal residue (Table 4.3) [7]. Gb3 is absent from p red cells and increased in P^k red cells [73,74]. Monoclonal anti-P^k react with Gb3 [75]. Several monoclonal anti-P^k were derived from mice immunised with synthetic glycoproteins containing the P^k-trisaccharide (Galα1→4Galβ1→4Glc) [42].

As the gene governing the P_1/P_2 polymorphism had been mapped to chromosome 22q13.2 [76,77], Steffenson *et al.* [45] carried out a database search within this region for a sequence homologous to that encoding an α-*N*-acetylglucosaminyltransferase in the hope of finding the gene for the P1 α1,4-galactosyltransferase. They identified a cDNA that encodes an α1,4-galactosyltransferase

(named α4Gal-T1), which catalyses the transfer of Gal from UDP-Gal to lactosylceramide (Gb2) to produce Gb3, the P^k antigen (Figure 4.1). It did not, however, convert paragloboside to P1 antigen, *in vitro*. When transfected with an α4Gal-T1 cDNA construct, Namalawa human lymphoblastoid cells, which have no endogenous α1,4-galactosyltransferase activity, strongly expressed P^k. The same gene, *A4GALT*, was identified by two other groups in the same year [46,77]. It comprises four exons [78], with the whole coding region in the fourth exon, and is located at chromosome 22q13.2 [45]. The GC-rich 5′-flanking region of *A4GALT* contains two silencer elements and a promoter element containing three Sp1 binding sites [79].

Homozygosity for a variety of mutations in *A4GALT* are responsible for the p phenotype by inactivating α4Gal-T1, preventing production of P^k and consequently P, and also of P1. The p phenotype is described in Section 4.10. The role played by *A4GALT* in P1 synthesis is described in Section 4.3.5.

Fabry disease is an X-linked, multisystemic, lysosymal-storage disease resulting from a *GLA* genetic defect leading to a deficiency of α-galactosidase A, which is responsible for the degradation of Gb3 [80]. Although there is an accumulation of Gb3 in capillary endothelial cells, testing of red cells for enhanced P^k expression has not been reported.

4.4.3 P^k on other cells

P^k (also known as CD77) has been detected on lymphocytes, granulocytes, monocytes, platelets, smooth muscle of the digestive track and urogenital system, and in other tissues [81]. P^k is also expressed on malignant cells and cell lines derived from them [75,82,83] and is a useful marker for Burkitt's lymphoma (BL) [83]. Of 40 different types of cells, BL cells showed the highest expression of *A4GALT* [84]. P^k (CD77) is a B cell differentiation antigen, with its expression largely restricted to germinal centre cells. Ligation of P^k to CD19, a B cell-restricted antigen, and their subsequent internalisation, appears to be involved in germinal centre B cell apoptosis [85]. Binding of monoclonal antibodies or verotoxin-1 (Section 4.12.1) to P^k induces apoptosis in BL cells through different pathways [86].

4.4.4 Anti-P^k

4.4.4.1 Alloanti-P^k

Alloanti-P^k is found, together with anti-P and -P1, in sera of p people. It can be separated from some of these sera by adsorption with P_1 cells [62]. These anti-P^k react equally strongly with P_1^k and P_2^k cells [62,87].

Anti-P^k is completely inhibited by HCF [3,65]. By inhibition of anti-P^k with fractions of HCF prepared by partial acid hydrolysis, and with oligosaccharides of known structure, Watkins and Morgan [72] concluded that anti-P^k was less demanding in its specificity than anti-P1. They found that the disaccharide Galα1→4Gal purified from the P_1^k glycoprotein of HCF inhibited anti-P^k, as did the P1 trisaccharide (Galα1→4Galβ1→4GlcNAc) and other oligosaccharides with terminal Galα1→4Gal. Anti-P1P^k isolated by addition of globoside to anti-PP1P^k was completely inhibited by P^k GSL (Gb3) [67].

4.4.4.2 Autoanti-P^k

Four examples of autoanti-P^k are recorded: two in patients with AIHA and two in patients with biliary cirrhosis [62].

4.4.4.3 Monoclonal anti-P^k

A rat monoclonal antibody (38.13) raised to a human Burkitt's lymphoma cell line (Daudi) [83], was shown to define Gb3 [75]. Tests against red cells demonstrated the expected anti-P^k specificity. Some other monoclonal anti-P^k resulted from immunising mice with synthetic glycoproteins containing the P^k trisaccharide (Galα1→4Galβ1→4Glc) [42] or with liposomes containing Gb3 glycolipid [88]. *A4GalT*-knockout mice were much more efficient at generating antibodies to Gb3 than conventional mice [88].

4.5 NOR (PIPK4) antigen and polyagglutination

NOR is a form of polyagglutination found in only two families, American and Polish, that appears to be inherited in a dominant manner [89,90]. Red cells of a total of nine individuals from two generations of each of the families were agglutinated by IgM antibody in 71–75% of ABO-compatible adult sera, but were not agglutinated by cord sera. The reaction of NOR cells with human sera was enhanced by papain and sialidase, but reduced by α-galactosidase treatment of the cells. NOR polyagglutination was completely inhibited by HCF and avian P1 substance, but NOR red cells had normal expression of P1 and P antigens.

Thin-layer chromatographs stained with a lectin specific for Galα1→3Gal (*Griffonia simpicifolia* IB4) revealed that NOR red cell membranes contained at least

two unique neutral glycosphingolipids (NOR1 and NOR2, Table 4.3) [90,91]. NOR antibodies (both mouse monoclonal and from human sera) are inhibited by the trisaccharide Galα1→4GalNAcβ1→3Gal (NOR-tri) and, to a much lesser extent, the disaccharide Galα1→4GalNAc (NOR-di) [92]. NOR1 is produced by extension of globoside (Gb_4Cer) by an α4-glactosyltransferase; NOR2 is produced by further extension of NOR1 by a β3-*N*-acetylgalactosaminyltransferase to NOR_{int}, which does not have NOR expression, and then by an α4-glactosyltransferase to NOR2 (Table 4.3, Figure 4.1) [93]. Weak cross-reactivity with Galα1→4Gal explains why the NOR polyagglutination is inhibited by P1 antigen [91]. Fourteen individuals with the NOR phenotype, from both families, were heterozygous for 631C>G in *A4GALT* (the P1PK gene), encoding Gln211Glu [94,95]. Transfection of 2102Ep cells with *A4GALT* led to expression of P^k on the cells; transfection with *A4GALT* containing the Gln211Glu mutation resulted in P^k and NOR expression [95]. It is likely, therefore, that Gln211Glu effects a change in enzyme activity, permitting transfer of Gal to GalNAc instead of, or in addition to, Gal. NOR antigen expression is, therefore, controlled by *A4GALT* and NOR antigen (P1PK4) belongs to the P1PK system.

4.6 P (GLOB1) antigen and anti-P

4.6.1 P antigen

P is found on all red cells except those of the rare phenotypes p and P^k (Table 4.2 and Sections 4.4 and 4.10). P is well developed at birth but, although P is expressed equally on cells from P_1 and P_2 adults [3], P_2 cord cells have a weaker expression of P than P_1 cord cells [96].

P was detected by flow cytometry with human alloanti-P on lymphocytes, granulocytes, and monocytes [23], although other antibodies failed to detect P on granulocytes, most peripheral blood lymphocytes, or fibroblasts [97,98]. P antigen is found on malignant cells and cell lines derived from them [82,97–99] and has also been detected on fetal liver, fetal heart, and placenta [12].

4.6.2 Biochemistry and biosynthesis

P antigen is globoside (Gb4) [7], which is lacking from P^k and p red cells, (or possibly present in trace amounts in p red cells) [72,73]. Globoside is the most abundant red cell membrane GSL with about 14×10^6 molecules per red cell [43,100] and represents Gb3 (P^k) with an additional non-reducing GalNAc residue (Table 4.3). Monoclonal anti-P was inhibited by the terminal trisaccharide of globoside, GalNAcβ1→3Galα1→4Gal, and by Galβ1→3GalNAcβ1→3Gal [101].

Okajima *et al.* [102] used an eukaryotic cell expression cloning system to isolate the cDNA encoding globoside synthetase, a β1,3-*N*-acetylgalactosaminyltransferase (β3GalNAc-T1). The cloned cDNA encodes β3GalNAc-T1, an enzyme that was previously considered to be a galactosyltransferase (β3Gal-T3) [103]. β3GalNAc-T1 synthesises P antigen from P^k by catalysing the transfer of GalNAc from UDP-GalNAc to Gb3 (Figure 4.1). The gene, *B3GALNT1*, is located at chromosome 3q25 [103] and consists of 5 exons, with only exon 5 encoding the enzyme [104]. It is widely expressed, with strong expression in brain and heart, moderate expression in lung, placenta, and testis, and low expression in kidney, liver, spleen, and stomach [102,103].

The P_1^k and P_2^k phenotypes, in which P^k is not converted to P, result from homozygosity for at least eight different mutations in exon 5 of *B3GALNT1*, including single nucleotide insertions, nonsense mutations converting a codon for an amino acid to a stop codon, and missense mutations encoding Asp150Gly, Arg216Ser, Glu266Ala, and Gly271Arg [104–108]. P^1/P^2 genotyping (Section 4.3.5) generally predicts P_1^k and P_2^k phenotypes, although there may be exceptions [106].

4.6.3 Anti-P

4.6.3.1 Alloanti-P

Anti-P is found in the serum of all P^k individuals and can be separated from serum of p individuals by adsorption with P_1^k or P_2^k cells [3,87], or by inhibition with HCF [72]. When complement is present, anti-P will haemolyse P_1 or P_2 cells. P antibodies are IgM and often also IgG, are usually reactive at 37°C, and can cause severe intravascular HTRs [109].

4.6.3.2 Autoanti-P and paroxysmal cold haemoglobinuria (PCH)

PCH is a form of AIHA occurring predominantly in young children following viral infections [110]. Sera from patients with PCH usually give a positive Donath-Landsteiner (DL) test; that is, the antibody binds in the presence of complement at 0°C and haemolyses the cells when subsequently warmed (reviewed in [111]). These biphasic haemolysins, or DL antibodies, generally have P specificity [112–114]. Sera from PCH patients react with P_1 and P_2 red cells, but not with p or P^k cells. Anti-P DL antibody is always IgG. Often the DL test is very weak in PCH and papain-treated red cells or acidified sera may be required before a positive result is obtained [115,116].

Very rarely the specificity of DL antibodies may anti-I, -i, -Pr [115] or 'anti-p' [96,117]. A few cases of AIHA, one with fatal consequences, were caused by IgG monophasic anti-P, haemolytic at temperatures between 20°C and 32°C [118–120]. P autoantibodies only detected in low ionic-strength solution (LISS) at room temperature did not give a positive DL reaction [121,122].

4.7 FORS1 and the Forssman glycolipid

Forssman (Fs) is a glycolipid, named after its eponymous discoverer [123], with a structure representing globoside (P) with an additional non-reducing GalNAc [124] (Table 4.3). Fs glycolipid is present on red cells of a variety of mammals, such as dog, sheep, horse, guinea pig, and mouse [125], but not usually on the red cells of humans and other primates. The gene encoding Fs-synthetase, which catalyses the addition of GalNAc to globoside (Figure 4.1), was initially cloned from a canine cDNA library [126]. A human orthologue, *GBGT1*, has seven exons and is located at chromosome 9q343 [127]. It is part of the GT6 glycosyltransferase family, which includes *ABO*, but transfection of *GBGT1* cDNA into COS-1 produced no Fs-synthetase activity [127] and *GBGT1* has been considered a pseudogene (Section 2.3.2.4).

A putative blood group A variant, named A_{pae}, was found in 1987 in three UK families [128]. A_{pae} red cells were agglutinated by 3 of 18 anti-A and 8 of 18 anti-A,B polyclonal reagents, but no monoclonal reagents, and by *Helix pomatia* lectin (see Table 2.21). The reaction with the lectin was inhibited by GalNAc. An A_{pae} propositus secreted H, but no A. Genotyping, however, revealed that A_{pae} individuals were homozygous for common *O* alleles [9].

In 2011, Hult *et al.* [9] showed that the A_{pae} determinant was not an A antigen, or related to ABO, but was Fs glycolipid. Consequently, Fs became FORS1, the sole antigen of the FORS blood group system. Monoclonal anti-Fs stained a distinct band in thin-layer chromatography of A_{pae} cell membranes, but not of membranes from other group O cells. Further structural analyses and characterisation of naturally occurring anti-Fs in donor sera confirmed that Fs was responsible for the A_{pae} phenotype.

Individuals with A_{pae}, that is FORS1-positive, red cells have 887G>A in *GBGT1*, encoding Arg296Gln. As glutamine is the residue at position 296 of the active animal Fs-synthetase, it is likely that Arg296 is responsible for inactivating Fs-synthetase in most humans and that Arg296Gln is responsible for activating the enzyme in A_{pae} individuals. Three-dimensional modelling of Fs-synthetase based on the crystal structure of the homologous ABO transferase, suggested that residue 296 of Fs-synthetase corresponds to His301 of the ABO transferase, which is important for catalytic activity [9].

4.8 LKE and anti-LKE

The P story was made more complex in 1965 when Tippett *et al.* [4] reported an agglutinin to a high frequency antigen in the serum of Mr Luke P, which behaved like anti-P because it did not react with p and P^k cells, but unlike anti-P because it also failed to react with the cells of about 2% of P_1 and P_2 people. In 1985, the monoclonal antibody 813-70, which defines the murine stage-specific embryonic antigen SSEA-4 [101], was shown to recognise the same red cell antigen as that detected by the antibody in the Luke serum [129]. The red cell antigen was given the symbol LKE.

4.8.1 Frequency and inheritance of LKE

The frequency of LKE– was about 2% in tests with the original Luke serum [4]. Tests on 950 English donors with MAb 813-70 gave the phenotype frequencies, LKE+ 98.84% and LKE– 1.16% [129]. From these the following gene and genotype frequencies were calculated:

LKE^+	0.8923	LKE^+/LKE^+	0.7962
LKE^-	0.1077	LKE^+/LKE^-	0.1922
		LKE^-/LKE^-	0.0116.

Similar frequencies of LKE– were found in Denmark (0.7%) with a human serum [130] and in the United States (1.2%) with 813-70 [69]; only four LKE– individuals were found among 2400 Scottish blood donors [131], an incidence for LKE– of 0.0017. LKE appears to be inherited as a Mendelian dominant character, though data from family studies are too few to be conclusive.

4.8.2 Biochemistry and biosynthesis

Recognition that a monoclonal antibody detecting SSEA-4, a murine stage-specific embryonic antigen [101], defined the red cell antigen LKE, demonstrated that

LKE is a globoseries antigen: monosialosylgalactosylgloboside (MSGb5), a globoside molecule with additional Gal and sialic acid residues [129] (Table 4.3). An LKE-active GSL was identified by high-performance thin-layer chromatography in the ganglioside fraction from LKE strongly positive red cells [69]. The molecular basis for the P+ LKE– phenotype is not known, but there are two prime candidate genes for biosynthesis of LKE from P: *B3GALT5*, which encodes a β1,3-galactosyltransferase (β3Gal-T5) that catalyses the synthesis of Gb5 from globoside (P) [132]; and *ST3GAL2*, which encodes an α2,3-sialyltransferase (ST3Gal-2) that catalyses the synthesis of MSGb5 from Gb5 [133].

4.8.3 Variation in strength of LKE

Variation in strength of reaction of LKE+ cells, classified as LKE+, LKEw, and LKE–, was observed with the Luke serum [4]. LKEw was more common in P_2 than in P_1, and more common in A_1 and A_1B than in O, A_2, A_2B, and B. Variation in the strength of LKE+ cells was also observed with the monoclonal antibody 813-70, but no effect of P_1 or A_1 was demonstrated [69,129], though in one study with the monoclonal antibody LKEw was more common in groups B and AB than in O, A_1, and A_2. The second human anti-LKE did not show any effect of P_1 or ABO groups on the strength of LKE+ reactions.

4.8.4 Development and distribution

Cord red cell samples react well with anti-LKE [129,131]. Monoclonal antibody 813-70 defines a mouse embryonic antigen, SSEA-4, which is also found on human teratoma cell lines [101], and is a marker for human embryonic stem cells [134] and mesenchymal stem cells [135]. LKE-active structures were detected in gangliosides isolated from platelets [136].

4.8.5 Involvement of other P antigens

LKE– individuals may be P_1 or P_2. Parallel testing with anti-P from P^k people and with monoclonal anti-P demonstrated that the strength of P on LKE– cells is the same as that on LKE+ cells [4]. P^k is more strongly expressed on P+ LKE– red cells than on P+ LKE+ red cells [68,69], with LKE– red cell membranes containing almost twice the quantity of Gb3 than LKE+ cells [69]. LKE– red cells have increased binding of verotoxins [69] (Section 4.12.1). Unlike P_1^k and P_2^k cells, which express P^k equally strongly, P_1 LKE– red cells have stronger P^k expression than P_2 LKE– red cells [131].

4.8.6 Anti-LKE

Six examples of human alloanti-LKE are known. The first was found in the serum of a black patient who had never been transfused [4]. The antibody was an agglutinin; the strength of reaction was increased by incubation at low temperature and by enzyme (trypsin, papain, or ficin) treatment of cells. When fresh, the Luke serum lysed papain-treated LKE+ red cells. The agglutinin was not inhibited by saliva or HCF. Five other examples of alloanti-LKE have been found, one present together with anti-P1 [130,131,137,138]. LKE+ babies of mothers with anti-LKE had no symptoms of HDFN [131,137], but one antibody, which was non-reactive in pre-warmed tests but haemolysed red cells in the presence of fresh human serum, was associated with post-transfusion haemolysis [138].

4.9 Sialosylparagloboside and PX2 antigen

An antibody reacting preferentially with p cells was specifically inhibited by sialosylparagloboside [139] (Section 4.11.1), paragloboside with a terminal sialic acid residue (Table 4.3). Sialosylparagloboside levels may be increased in p cells because a blockage in the synthesis of both P^k and P1 results in increased quantities of precursor glycolipids for other biosynthetic pathways (Figure 4.1).

PX2 represents paragloboside with an additional β1→3GalNAc residue [140,141] (Table 4.3). Considerably enhanced quantities of PX2 and its sialylated derivative are present on p phenotype red cells. It is possible that the product of *B3GALNT1*, the same enzyme as that responsible for synthesis of P, catalyses synthesis of PX2 in p cells, where its usual substrate, P^k is absent [141]. Weak reactions with p red cells by most antibodies produced by individuals with the P^k phenotype are probably explained by the presence of anti-PX2 in addition to anti-P [61].

4.10 p Phenotype and anti-PP1P^k

In 1951, Levine *et al.* [142] described an antibody in the serum of a woman with gastric carcinoma, which reacted with all cells except for her own and those of her sister. The antibody was called anti-Tj^a (T for tumour, J for the patient's name). Sanger [2] proposed that Tj(a–) be

called p following her observation that six unrelated Tj(a–) individuals were P_2. Red cells of the p phenotype lack P1, P^k, P, and LKE antigens, although they express enhanced levels of PX2.

4.10.1 Frequency and inheritance of p phenotype

The p phenotype is very rare. Race and Sanger [62] calculated a frequency of 0.0024 for the *p* gene, giving p a phenotype frequency of 5.8 per million people of European origin. The p phenotype is more common in Japan, but screening of over 1 million Hong Kong Chinese revealed no example of p [143]. In the Vasterbotten country of northern Sweden, eight p individuals were found from screening 40 149 donors with anti-$PP1P^k$, a phenotype frequency of about 141 per million [144].

Information from many families with p propositi supports recessive inheritance of *p* [62,145–148], as does the high consanguinity rate.

4.10.2 Molecular genetics of p phenotype

The p phenotype results from homozygosity (or compound heterozygosity) for various missense mutations and nonsense mutations in *A4GALT*, the gene that encodes the enzyme responsible for converting lactosylceramide to P^k (Gb3) and paragloboside to P1 (Figure 4.1) [45,46,104,106,108,149–153]. Most p Swedes are homozygous for 548T>A, Met183Lys or 560G>A, Gly187Asp, with the former as the predominant allele [45,46,104,150]. The most commonly encountered mutations in p Japanese appear to be 752C>T, Pro251Leu [46,104], a triple nucleotide deletion (241_243delTTC) resulting in deletion of Phe81 [104,149,150], and a single nucleotide insertion (1026_1029insC) resulting in a frameshift, disruption of the stop codon, and an additional 92 amino acids [104,149,152]. The latest published list of mutations is in Hellberg *et al.* [153] and they are also listed in dbRBC [108]. Transfection experiments for many of these mutations have shown that they resulted in either no enzymatic activity or only marginal activity, *in vitro* [46,152,153]. P^1/P^2 genotyping (Section 4.3.5) demonstrated the expected linkage between the *p* mutation in *A4GALT* and the P^1/P^2 polymorphism in the same gene: of 20 distinct mutations in 22 different alleles, 11 were associated with P^1 and 11 with P^2 [106]. In all Amish p samples, for example, *A4GALT* 299C>T (Ser100Leu) was linked to P^1.

P synthetase was present in cultured fibroblasts and B-lymphocytes from p individuals [154,155], but could not synthesise globoside in the absence of its acceptor substrate, P^k, explaining the absence of P.

4.10.3 Biochemical effects of p phenotype

Despite the lack of two abundant GSLs, Gb3 and globoside, p red cells appear normal in behaviour and in morphology. Red cells with p phenotype have increased quantities of lactosylceramide and other complex glycolipids [44,73,154], including the PX2 antigen and its derivatives [140,141]. Kidney contains high levels of extended globoseries compounds. Kidney obtained at autopsy from a group A, p phenotype individual, had enhanced levels of lactosylceramide, no Gb3 or globoside, and no Type 4 A (globo-A) chain structure [156] (Section 2.2.2).

4.10.4 Antibodies in serum of p individuals

All p people have antibody in their serum, generally called anti-$PP1P^k$, which agglutinates and/or haemolyses all red cells except those of the p phenotype. Adsorption of p serum with P_2 cells to remove anti-P leaves activity against P_1, but not P_2, cells [2]. Adsorption with P_1^k cells removes anti-P1 and -P^k leaving anti-P [3]; surprisingly, adsorption with P_2^k cells has the same effect [87]. Specific anti-P^k can be made from only some anti-$PP1P^k$ sera. Tippett [157] adsorbed sera from 47 p people with P_1 cells, but only succeeded in making anti-P^k from less than half of these sera, and with those sera continued adsorption with P_1 cells removed or weakened the anti-P^k. Inhibition tests on four p sera with various GSLs indicated that, after inhibition of anti-P with globoside, most of the remaining antibody is cross-reacting anti-$P1P^k$ [67]. This offers an explanation for the inability to isolate anti-P1 from anti-$PP1P^k$ by adsorption with P_2^k cells. Anti-$P1P^k$ was mostly IgG [67], in contrast to the anti-P1 of P_2 people, which is usually IgM. The anti-P component in the sera of two p individuals was IgM and cross-reacted with Forssman antigen; the rest was IgG and specific for globoside [158]. Most of the anti-P^k in these sera was IgG.

IgG and IgA activity to P, P1, and P^k carbohydrate structures, but IgM activity to only P1 and P^k structures, was detected in p sera by radioimmunoassay. All but one of 13 p sera contained IgG3 antibodies to P, P1, and P^k oligosaccharides; some also contained IgG1 and/or IgG2 antibodies, but none contained IgG4 [159].

Anti-$PP1P^k$ is capable of causing rapid removal of transfused cells and severe HTRs [109]. Injection of the original p individual with 25 ml of incompatible red cells resulted in a severe HTR [142]. Anti-$PP1P^k$ as a

potential cause of early abortion and HDFN is discussed in Section 4.13.

4.10.5 p Phenotype and cancer

The original p phenotype was in a woman with gastric carcinoma [142]. She was treated by subtotal gastrectomy, which was a complete success and in the 22 years until her death from unrelated causes there was no evidence of tumour recurrence or metastasis [160]. Unlike her red cells, the tumour expressed P system antigens [161], which led Levine [160] to propose his theory of 'illegitimate' antigens, antigens present on tumours contrary to the genetic constitution of the patient. Moreover, Levine suggested that her anti-PP1P^k had prevented further growth of the tumour.

4.11 Other P antibodies

4.11.1 'Anti-p'

Several alloantibodies have been described that react strongly with p cells and much more weakly, or not at all, with P_1, P_2, and P^k cells [96,117,162]. These antibodies differed slightly in their serological characteristics. One of them was an agglutinin and biphasic haemolysin, which reacted very strongly with p cells, less strongly with P_2 and P_2^k cells, and much less strongly with P_1 and P_1^k cells [162]. The red cell antigen recognised by this antibody was destroyed by sialidase treatment and was identified as sialosylparagloboside [139]. P2X antigen is also responsible for enhanced reactivity of some antibodies with p red cells [61,140,141] (Section 4.9, Table 4.3).

4.11.2 Anti-IP1, -I^{T}P1, -I^{T}P, and -IP

Anti-IP1 behave as anti-P1 except that they are non-reactive with P1 cord or P1 adult i cells [163]. Anti-I^{T}P1 was identified in a Melanesian [164]. Bithermic anti-I^{T}P behaved as anti-I^T, apart from its failure to agglutinate p cells [165]. Anti-IP, together with anti-IP1, was found in a patient with unusual P and I antigens [166].

4.12 P antigens as receptors for pathogenic micro-organisms

4.12.1 Pathogenic bacteria and their toxins

Escherichia coli is responsible for most recurrent urinary tract infections. Uropathogenic *E. coli* attach to uroepithelial cells before they invade them. Adherence is achieved by lectin-like structures called adhesins, encoded by *pap* genes and located on P fimbriae on the bacterial surface. Isolates of uropathogenic *E. coli* expressing *pap*-encoded adhesins bind to globoseries glycoconjugates containing the disaccharide Galα1→4Gal, including P^k, P1, P, LKE (MSGb5), DSGb5, and globo-A (Type 4 A) (Table 4.3) (reviews in [167,168]). Red cells of the p phenotype are not agglutinated by pyelonephritogenic *E. coli* fimbriae and the bacteria have impaired adhesion to uroepithelial cells from p individuals [169]. Uroepithelial cells from ABH non-secretor women have enhanced adherence to uropathogenic *E. coli* compared to those from secretors [170]. *E. coli* R45 binds to MSGb5 (LKE) and DSGb5 (Table 4.3), structures that are selectively expressed by epithelial cells of non-secretors, presumably as a result of sialylation of the galactosylgloboside precursor, which is fucosylated to globo-H (Type 4 H) in secretors [170].

Some strains of enterohaemorrhagic *E. coli* produce enterotoxins, called verotoxins, which are highly homologous to the Shiga toxin produced by *Shigella dysenteriae*. These verotoxins are associated with diarrhoeal illness and other diseases including haemolytic uraemic syndrome (HUS) [168,171]. P^k antigen is a ligand for VT1 and VT2 [171,172]. Chinese hamster ovary cells that do not express P^k and are resistant to Shiga verotoxin become susceptible to the toxin following transfection with P^k synthetase cDNA [78] and P^k synthetase knockout mice are resistant to doses of verotoxins 100 times higher than those required to kill wild-type mice [173]. Verotoxins induce apoptosis through binding to P^k on megakaryoblasts, which could be a cause of thrombocytopenia in HUS [84], and on Burkitt's lymphoma cells and other malignant cells [86,172,174], which might have therapeutic potential. Verotoxin-induced cytotoxicity and transmembrane signalling require that Gb3 (P^k) is situated within a lipid raft [171].

4.12.2 Parvovirus B19

P antigen is a cellular receptor for parvovirus B19 [175], a human pathogen that is highly tropic to bone marrow and only replicates in erythroid progenitor cells. B19 is the cause of fifth disease, a common childhood illness, and occasionally more severe disorders of erythropoiesis, particularly in immunocompromised patients [176]. B19 empty capsids agglutinate P_1 and P_2 red cells, but not P^k or p cells. The cytotoxic effect of B19 on erythroid colony formation in culture is prevented by sensitising the cells with monoclonal anti-P, but not with anti-P1 or -P^k; there is no cytotoxicity when cells are derived from a p marrow

[175,177]. P antigen is necessary, but not sufficient, for attachment and entry of B19 in erythroid progenitors [178]. Mature red cells express large quantities of P antigen, yet are not invaded by the virus. Activated α5β1 integrin [179] and the Ku80 autoantigen [180] have both been proposed as co-receptors for B19. Individuals with the p phenotype appear to be naturally resistant to B19 infection [177].

4.12.3 HIV-1

GSLs within cholesterol-rich lipid rafts play a vital part in the infection of host cells by HIV-1. Interactions between GSLs, CD4, and the HIV envelope glycoprotein gp120 may facilitate the migration of the virus to its co-receptors CCR5 or CXCR4 and to membrane fusion (reviews [171,181]). Peripheral blood mononuclear cells (PBMC) from patients with Fabry disease, which have enhanced P^k expression (Section 4.4.2) are resistant to infection by the R5 strain of HIV-1 [182]. PBMCs from individuals with the P_1^k phenotype, where P^k is heavily expressed, are highly resistant to R5 and X4 HIV-1 infection, whereas PBMCs from p individuals are up to 1000 times more susceptible to HIV-1 infection [183]. A competitive inhibitor of α-galactosidase A induced P^k accumulation in HIV-infectable cell lines and decreased susceptibility to HIV-1 infection, whereas a glucosylceramide synthase inhibitor, which depletes cells of P^k, substantially increased susceptibility [184]. P^k, therefore, appears to afford protection against HIV-1 infection, possibly through disruption of the organisation of the lipid rafts. A synthetic P^k mimic (FSL-Gb3), which is non-toxic and completely soluble in aqueous solution, prevents HIV-1 infection by direct inhibition of virus and inhibition of viral entry, and so might provide a therapeutic approach for HIV/AIDS [185].

4.13 The association of P antibodies with early abortion

The incidence of habitual spontaneous abortion is significantly higher in women with the p phenotype than in most of the population. Many women with the p phenotype have been ascertained through habitual abortion, though other p women have several live children. Abortions occur characteristically in the first trimester; embryos that survive this critical period usually develop to healthy babies. Most P_1 or P_2 babies of p mothers have no sign of HDFN, although there are a few reports of mild HDFN [145,186].

It is almost certain that anti-PP1P^k in the sera of p women is the cause of the abortions [187], and the anti-P component is the most likely culprit. Habitual spontaneous abortion has also been reported in women with the P^k phenotype: a P_2^k Japanese woman and a P_1^k Kuwaiti woman suffered four and 13 early abortions, respectively [188,189]. Neither had any live children, but in both a procedure of therapeutic plasmapheresis begun at the fifth or sixth week of pregnancy was rewarded by a live birth. Neither baby required any treatment other than phototherapy. In the Japanese case, autologous plasma was returned to the mother after *ex vivo* removal of anti-P by adsorption with donor red cells [188]. Plasmapheresis procedures have subsequently been used successfully for p women with a history of multiple abortions and no live children [159,190–193]. Other children born to P^k mothers have been reported to have no sign of HDFN or only mild HDFN [63,194].

Glycosphingolipid fractions prepared from 12- and 17-week-old fetuses obtained following spontaneous abortions in two p women had only trace amounts of P and P^k antigen activity, whereas the placental fractions had high P and P^k activity. IgG3 antibodies from the serum of one of the p mothers bound strongly to placental glycolipids, but not to glycolipid fractions from the fetus [195]. IgM, IgG (mostly IgG3), and IgA antibodies, strongly reactive with globoside (P antigen) isolated from placenta, were present in the serum of the Kuwaiti P_1^k woman [196]. The primary target for antibodies in p and P^k aborters appears, therefore, to be the placenta and not the fetus.

An unusual antibody was reported in the serum of 'habitual aborters' (pregnant women who threatened to abort for at least a second time) in Perth, Western Australia [197]. This antibody haemolysed, but did not agglutinate, all P_1 and P_2 red cells, but did not haemolyse or agglutinate p cells. The patients were of normal P1 groups. The haemolysin was only present at the time of the threatened abortion [197]. The haemolytic activity did not appear to be complement dependent [198]. Vos [197–200] exhaustively studied these puzzling patients, looking for an environmental or immunological cause for the phenomenon, but no explanation was forthcoming.

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