Red cell antigens: Structure and function

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Abstract:

Landsteiner and his colleagues demonstrated that human beings could be classified into four groups depending on the presence of one (A) or another (B) or both (AB) or none (O) of the antigens on their red cells. The number of the blood group antigens up to 1984 was 410. In the next 20 years, there were 16 systems with 144 antigens and quite a collection of antigens waiting to be assigned to systems, pending the discovery of new information about their relationship to the established systems. The importance of most blood group antigens had been recognized by immunological complications of blood transfusion or pregnancies; their molecular structure and function however remained undefined for many decades. Recent advances in molecular genetics and cellular biochemistry resulted in an abundance of new information in this field of research. In this review, we try to give some examples of advances made in the field of 'structure and function of the red cell surface molecules.'

Key words:

Adhesion molecule, blood group antigens, complement, enzyme activity, GPI-glycophosphatidylinositol

Landsteiner[1] and his colleagues[2] demonstrated that human beings could be classified into four groups depending on the presence of one (A) or another (B) or both (AB) or none (O) of the antigens on their red cells. Group A individuals were further subdivided into A_1 and A_2 on the basis of the strength of the antigen, the former being stronger.[3] Regular occurrence of ABO antibodies with reciprocal relation of antigen on red cells and the antibody in serum not only helped in confirmation of the ABO blood group of an individual but also helped in detection of weaker variants of A/B on red cells by absence of corresponding antibody in the serum. Innumerable weaker variants have been recognized and classified on the basis of reaction pattern with different reagents as well as salivary secretor status.[4]

In this review, we try to give some examples of advances made in the field of 'structure and function of the red cell surface molecules.'

In order to look for further human difference,[5] injected rabbits with O group blood and selective absorption of immune rabbit sera left with the antibody for the antigens to be designated as P, M and N. The antithetical association of the M and the N grouped them into three phenotypes M, MN and N. The system was expanded later by the discovery of closely associated antigens S and s and various satellite antigens.[4]

Another set of animal experiments[6] immunized rabbits and guinea pigs using the red cells of Rhesus monkeys. The resultant anti-Rhesus sera were tested with human blood to find out antigenic resemblance between the two closely related species. The antigen detected by the antibody was referred to as Rh (Rhesus) factor, which was present in almost 85% of the human white. Clinical significance of the Rh factor was realized by retrospective analysis of a case with erythroblastosis fetalis caused by anti-Rh antibody developed in the mother by immunization through pregnancy.[7] Many examples of human antibodies were then recognized, and many of them were not identical but directed to the antigen closely related to the Rh. The original Rh antigen was referred to as D and the related ones as C and E; the antithetically related antigens were called 'c' and 'e.' So far there is no evidence of antigen 'd' reported in the literature.

Technical revolution in blood group serology revealed various examples of blood group antibodies which did not have a property of giving direct agglutination but agglutinated red cells with the help of high protein medium or use of proteolytic enzymes to modify the red cell membrane.[8,9] The classical development of antiglobulin test[10] helped in identifying many more examples of red cell antibodies as a cause of transfusion reaction and neonatal jaundice. It appears that there was little, but significant, progress in the field of blood group serology until the use of antiglobulin test in 1945. The field was then significantly grown with a straight peak in

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the graphical representation [Figure 1]. Many of the blood group antigens formed well-defined systems and yet several other examples await their precise placement and are therefore listed as public or private antigens depending on the frequency of the antigen in general population.

The number of the blood group antigens up to 1984 was 410.[11] In the next 20 years, there were 16 systems with 144 antigens and quite a collection of antigens waiting to be assigned to systems, pending the discovery of new information about their relationship to the established systems.

Since the discovery of Bombay (Oh) phenotype in 1952,[12] the ABH blood group system has been better understood for the precise structure of these antigens, biosynthesis and genetics. Levine et al.[13] reported that Bombay phenotype is not always genetically group O but is a result of the homozygous suppressor genes xx, which suppress the action of normal A/B and secretor genes present in these individuals. Watkins and Morgan[14] in 1959 suggested a mechanism for the biosynthesis of ABH antigen due to stepwise gene action and interaction of those genes resulting into the development of ABH antigens on red cells and saliva. All cases of Bombay phenotype, including the cases with known suppression of A or B antigens, were tested with battery of anti-A, anti-B and anti-H (Oh) sera. No agglutination nor any antibody absorptions and elutions were obtained in these cases, suggested as typical Oh cases.

On the other hand, Para Bombay cases encountered in India and other cases reported in literature showed weaker reaction with a number of anti-H reagents of Oh and chicken origin. Comparative tests with cord and adult O cells suggested that weak H antigen in these variants was predominantly fetal type. Antigens in saliva show distinct features. All typical Oh cases are nonsecretor, while Para Bombay cases are classified as either nonsecretors or secretors – Bhatia et al.[15] Although H and h are encoded by a gene on a different chromosome from ABO, the Hh blood group system is subsumed in the ABO system, H being a precursor of A, B blood group.[16,17]

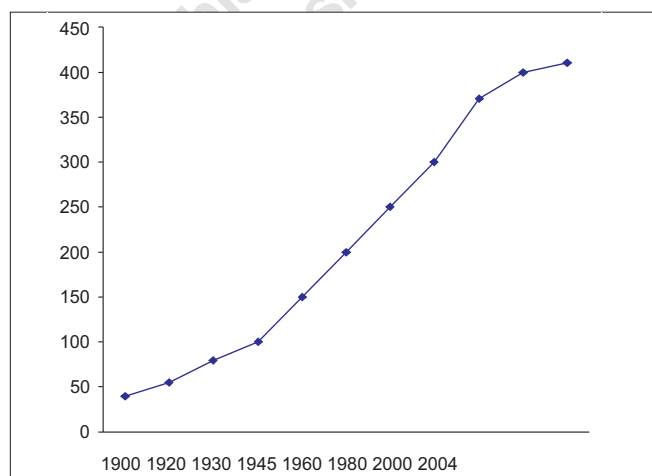The Lewis system is a system of soluble antigens present in saliva and plasma,[18] and red cells acquire their Lewis phenotype by adsorbing Lewis substances from the plasma.[19]

The Lewis phenotype of the red cells is influenced by the ABH secretor status (although the Lewis genes and secretor genes are inherited independently): subjects who inherit Le will have the red cell phenotype $Le^{(a+,b-)}$ if they are nonsecretors (se se), but the phenotype $Le^{(a-,b+)}$ if they are secretors – Grubb 1951, Ceppellini 1955.[17] Cord red cells do not react with anti-Le^b and are not agglutinated by anti-Le^a. However, using the IAT, Le^a can be demonstrated on the cells of about 50% of cord blood samples.[20]

The weak reactions of the red cells of newborn infants seem to be due to the very low concentration of Lewis glycolipids in the plasma.

Lewis antibodies, particularly anti-Le^a, can cause rapid destruction of small volumes of injected washed incompatible red cells. The only risk arises if Le^{a+} red cells of group O, which have more Lewis antigens than A or B cells, are selected for a patient whose serum contains potent anti-Le^a; in these circumstances, Le^{a-} red cells should be transfused.[21]

Lewis antibodies are not known to cause hemolytic disease in the newborn, because Lewis antibodies are predominantly IgM.

Almost all individuals are either P_1 (about 75% of the English population) or P_2; P_2 simply implies P_1 negative: there is no P_2 antigen; P_2 subjects frequently have anti-P_1 in their serum as a cold agglutinin, which is only occasionally active at 20°C or higher.

Among P_1 subjects, there is considerable variation in the strength of the P_1 antigen, and this variation is inherited.[22]

The P antigen is the receptor for parvovirus B19, and subjects who lack P are naturally resistant to infection with the virus.[23]

When measured by fluorescence flow cytometry, the distribution of P_1 and P antigens on red cells was shown to be heterogeneous, the amounts varying from cell to cell within a given red cell population.[24]

Echinococcus cyst fluid scolics in hydatid cyst fluid contain P_1 and occasionally stimulate the production of anti-P_1 in humans with hydatid disease.[25] Pegeon red cells and serum contain an antigen similar to P_1 but not identical with human P_1 Brocteur.[25]

P_1 and P were found on platelets and their distribution was also heterogeneous.[24] P_1 and P are present on lymphocytes and fibroblasts. P^k antigen is present on fibroblasts of normal P_1 and P_2 people.[26]

I and i are not antithetical; instead, the antigens are related structures. It can be argued that this is not a blood group system, because the i antigen is the precursor to I, much the same as H is to A or B. There is also biochemical evidence that I and i are precursors of ABH antigens. The two antigens I and i are high-frequency antigens inversely proportional each other. At birth the newborn's red cells have a large amount of i antigen with almost undetectable I antigen; I increases as i decreases, until about 18 months, when the red cells will test it with little detectable i antigen. A few rare adults continue to have almost

Figure 1: A diagrammatic representation of the number of red cell antigens known, year by year

undetectable I antigen levels.[27]

Antigens of the Ii systems are heterogeneous, and the amount of I antigen on the red cells of different individuals varies. The I antigen is poorly developed on cord cells, but there is usually a trace of I antigen. I and i substances are found in many biological fluids. In soluble form, they are present in serum, saliva, milk, amniotic fluid, urine and ovarian and hydatid cyst fluids.[28] Ii-deficient phenotypes have been described, and dominant inheritance pattern was suggested. The Ii phenotype, characterized by weak reactions with both anti-I and anti-i, does pose many questions.[29]

There is no report indicating the plasma level of ABH and I antigen in leukemia. In our series, though the red cell ABH was found to be reduced, the plasma level for ABH and I antigens was elevated in leukemia.[11]

In the Japanese population, the genes coding for i and for autosomal recessive congenital cataracts are linked.[30]

The importance of most blood group antigens had been recognized by immunological complications of blood transfusion or pregnancies; their molecular structure and function however remained undefined for many decades.

Oligosaccharides that carry ABH antigens can be attached to either protein (glycoprotein), sphingolipid (glycosphingolipid) or lipid (glycolipid) carrier molecules.

Glycoproteins and glycosphingolipids carrying A or B oligosaccharides are integral parts of the membranes of red cells, epithelial cells and endothelial cells and are also present in soluble form in plasma. Glycoproteins secreted in body fluids such as saliva contain molecules that may, if the person possesses an Se gene, carry identical A and B oligosaccharides.

A and B oligosaccharides unattached to carrier protein or lipid molecules are also found in milk and urine [Figure 2].

The glycolipid antigens of these four systems are essentially derived from the same precursor by sequential addition of sugars to similar oligosaccharide chains. There are many structural similarities, and a single molecule may have both ABH and Lewis

specificities.[31] A simplified version of the biosynthetic pathway is shown in Figure 3. It is apparent that any influence on the precursor substance could affect the expression of ABH, Ii, P or Lewis and this may, in part, be the explanation of observed inhibition of P_1 and i antigens by In (Lu). The P blood group antigens (broken line) are related to the other antigens but are not located on the same structures. The Ii genes may code for an intermediate product that is a precursor of the H antigen and therefore of A and B antigens. The sequence starting from the cell membrane is P, I, H, AB, but the branched glycosphingolipids are folded in their natural state, which narrows the distance in between.

Four H antigen structures have been identified: H_1 and H_2 are not branched whereas H_3 and H_4 are branched.[32] Because H is the substrate for A and B antigens, there are, in turn, four A and B structures. The ABH of the fetus and newborn is typically unbranched: A^a, A^b, B^I, B^{II}, H_1, H_2 with i antigen. Larger concentrations of branched ABH antigens are found on adult red cells: A^c, A^d, B^{III}, B^{IV}, H_3, H_4, with I antigen. A maturation process may be involved.

Synthesis of plasma antigens (dotted line) only involves type I chain precursor. The level of A or B antigens in plasma is related to the ABO type, secretor status and Lewis phenotype. A secretor who is A_1 Le (a⁻ b⁻) will have a higher level than one who is A_2 Le (a⁻ b⁺). Secretors generally have greater amounts of ABH substance than do nonsecretors of the same blood type.

Specificity of the antibodies in these systems varies. Some (such as anti-A, anti-B, anti-H, anti-Leᵃ and anti-P_1) react with the immunodominant sugar or a complex containing this sugar, at or near the terminal end. Others, such as anti-i, react with repeating linear sequences – both internal and terminal. With branching of the structure, antibody recognition may become more complex, with some antibodies specific for different branches or against all branches; this is seen with anti-I.

Given the close relationship to the structures, it is not surprising to find antibodies that react only with red cells that have a certain combination of antigens from the four systems. The most common of these is anti-IH, which requires the presence of both H and I antigens to react. There are many other interaction antibodies, such as anti-IP_1 and anti-A_1Leᵇ.[33]
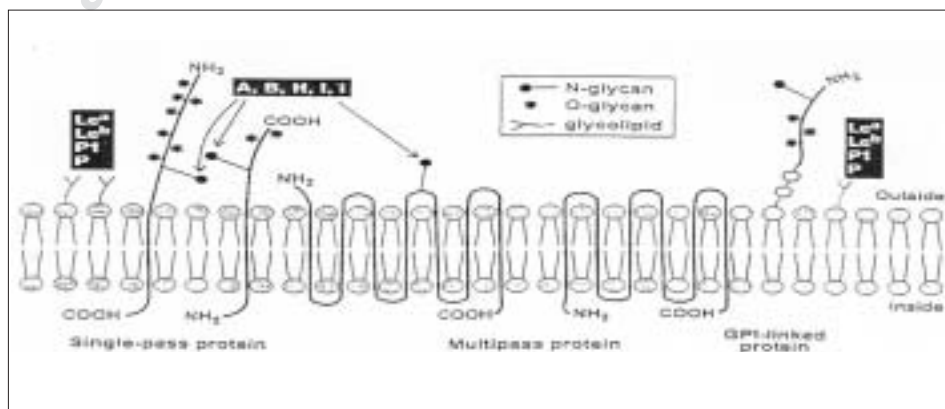

Figure 2: Schematic representation of the red cell membrane showing antigen-bearing glycosylation of proteins and lipids GPI = glycophosphatidylinositol.
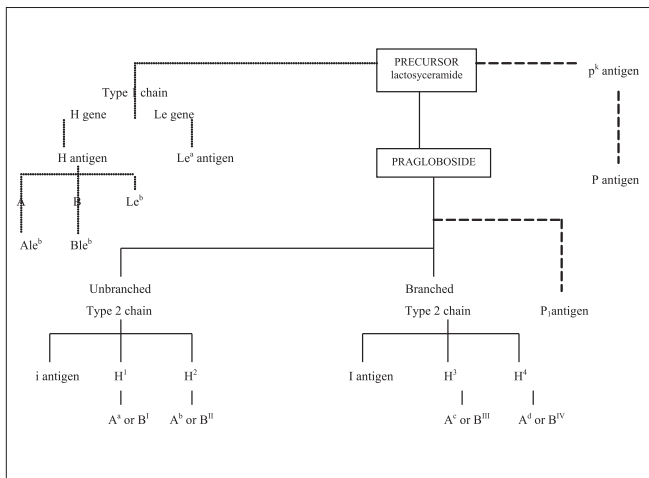
Figure 3: Biosynthesis of antigens of the ABO, Ii, P and Lewis systems (Watkin W.M. 1980)

Red cell antigens which are proteins (e.g., Rh) are direct products of genes, but those which are carbohydrates (e.g., ABO) are determined indirectly by enzymes (transferases), which are gene products; these enzymes transfer the appropriate sugar determining specificity on to a structure whose synthesis may be determined by one or more unrelated genes. In most cases, there appears to be a simple correspondence between genes and antigens, so that if a person inherits a given gene, the antigen can be detected on the red cells. It is not uncommon for one gene to interfere with the expression of another, carried on a different chromosome. For example, the expression of Le is modified by A and B and that of Lu may be modified by inhibitor genes.[33]

RH antigen

The highly polymorphic Rh blood group system consists of at least 45 independent antigens.[34] The antigens of the Rh blood group system are located on two proteins. RhD carries the D (Rh1) antigen and Rh CE carries the C, c, E and e (Rh2 to Rh5) (Rh2=C, Rh3=E, Rh4=c, Rh5=e) antigens. On the other hand, various genotypes can be deduced and the most probable genotype can be singled out from these possibilities using the relative frequency of various haplotypes in a particular population which the person concerned belongs to. Table 1 shows eight Rh gene complexes among the RhD+ and RhD- phenotypes,[35] with Rh-associated glycoprotein (RhAG), previously known as RH50. This Rh complex is tightly linked to the cytoskeleton. Several additional proteins, such as CD77, LW and the Duffy glycoprotein, are associated with the Rh complex but not necessary for Rh expression. The membrane expression of Rh depends on functional RhAG. Mutation in RhAG could be shown to underlie

Table 1: Showing eight Rh-gene complexes among the Rh D+ and Rh D- subjects in the RBC membrane, the Rh proteins form a complex

RhD+		RhD-	
Gene complex	Short notation	Gene complex	Short notation
CDe	R^1	cde	r
cDE	R^2	Cde	r'
cDe	R^0	cdE	r''
CDE	R^z	CdE	r^y

the regulator form of the Rh null phenotype characterized by lack of all Rh antigens.[36]

The genes encoding the Rh proteins (RhD and RhCE) are highly homologous and adjacent on the short arm of chromosome 1. The gene encoding RhAG is located on the short arm of chromosome 6. After the A and B antigens of the ABO system, the D antigen has greater immunogenicity than virtually all other red cell antigens; more than 80% of D-negative persons who receive a D-positive transfusion are expected to develop anti-D.[33]

Among the other common Rh antigens, c and e are more potent immunogenes. Antigens C, E and G prove themselves immunogenic mostly in partnership with D antigen. Antigenic sites on the red cells vary considerably depending upon the genetic constitution as to the dosage effect of a particular gene, type of the genes accompanied in the gene complex and the antisera used. For example, Huges-Jones et al.[37] found c antigenic sites on cc cells to be between 70,000 and 85,000 and on cC cells between 37,000 and 53,000. On the other hand, Masouredis et al.[38] observed c antigenic sites to be 31,500 on cde/cde cells, while there were 24,000 sites on cDE/cDE cells. The compound antigenic nature of the common Rh antigens other than D is seen as if they are of the cis gene product, i.e., they are expressed when one each of the C, c and E, e allelic pairs is inherited as a genetic unit on the same chromosome and not expressed if the genes are carried on opposite chromosomes in the trans position. There have been four such compound antigens recognized through the specific antibodies found against each, viz., ce (also called f, Rh:6), Ce (also known as rh, Rh:7) CE (Rh:22), cE (Rh:27). Of these, ce has its variant called ce^s (also called V, hr^v or Rh:10), frequently occurring among the African race. Antibodies to the compound antigens may serve as a useful tool in confirming the Rh genotype of an individual showing several possibilities emerging out of an observed phenotype. For example, the red cells from an individual showing a phenotype CcdEe using conventional subtyping antisera can have two possibilities as genotypes CdE/cde (r^{y}r) or Cde/cdE (r'r''). However, the red cells reacting with anti-ce (-f) or anti-CE and not with anti-Ce (-Rh$_i$) or anti-cE would confirm an actual genotype of that person to be CdE/cde.

Antigen G (rh^G, Rh:12) is found on almost all D$^+$ or C$^+$ red cells. A rare blood with G-positive and D-negative has been recorded. The cells having G but lacking both C and D are expressed as r^G. Antigens C^w (Rh:8), C^x (Rh:9) and MAR (Rh:51) represent alleles at the C-c locus of which C^w and C^x are rare antigens believed to be antithetical to a high frequency antigen MAR. Among the variant antigens encoded by alleles at E-e locus, E^T (Rh:24) was found among the Australian aborigines and is thought to be a partial E antigen. Similar, but not necessarily identical, examples of partial E have been encountered in recent times, which suggest that the Rh antigen E, as D, comprises several types of partial E antigens. E^W (Rh:11) is defined by specific anti-E^W. The hr^s (Rh:19) and hr^B (Rh:31) represent partial e antigens usually found in the population of African origin; V (ce^s, [Rh:10]) and VS (e^s, [Rh:20]) and R$^{=N}$ are further antigens encountered among the Black population.[39]

The phenotype described as Rh$_{null}$ may be produced by at least two different genetic mechanisms. In the more common regulator

type of Rh_{null}, mutations occur in the RHAG gene that result in the complete absence of the core Rh complex (Rh polypeptides and RHAG) that is necessary for the expression of Rh antigens. Such persons appear to transmit normal RHD and RHCE genes to their offspring in a manner roughly analogous to that in which A or B transferases are transmitted by people of the Bombay phenotype. In some cases, parents or offspring of people with the regulator type of Rh_{null} show overall depression of their Rh antigens known as Rh_{null}. The other form of Rh_{null}, the amorph type, has a normal RHAG gene; however, there is a mutation in each RHCE gene together with the common deletion of RHD. The amorph type of Rh_{null} is considerably rarer than the regulator type. Parents and offspring of this type of Rh_{null} are obligate heterozygotes for the amorph.[40]

Spontaneous or drug-induced autoimmunity may occur however in RhD positives. Rh null (—/—) phenotype individuals are characterized by mild hemolytic anemia with stomatocytosis and spherocytosis.

Maternal immunization usually results from the transplacental passage of fetal RBCs that express paternally inherited antigens into the maternal circulation. The most frequent cause in severe HDN is anti-D. This is due to the high immunogenicity of D and is related to the volume of fetal RBCs that leak into the maternal circulation; other red cell antigens are less immunogenic than D.[41,42]

D-negative women may become immunized by fetal D+ RBC that cross the placenta. The occurrence and volume of fetomaternal hemorrhage (FMH) is greatest at parturition, that in our recent study.

In our recent study, we quantified fetal cells in the maternal circulation and evaluated transferred antigens from fetus to mother by anti-D and anti-HbF flow cytometry. We measured fetal hemoglobin to evaluate all blood group incompatibility[43] and quantified RhD antigen for RhD antigen incompatibility. Our results showed that although the methodological flow cytometric approach using anti-D is applicable only to the clinical situation with RhD antigen incompatibility, yet it is significantly much more accurate than HbF labeling.[44]

LW antigens

The original anti-Rh produced through immunization of rabbits and guinea pigs was shown at that time as having the same specificity as anti-D; however, later on it was proved that it had a different specificity.[45] LW glycoprotein appears to be part of the Rh membrane complex. However, LW, being located on chromosome 19, is genetically independent of Rh locus that is situated on chromosome 1. The human version of anti-LW was also detected and later on it showed negative reactivity with Rh null cells.[35]

The antigens are denatured by sulphydryl reagents such as DTT but are unaffected by papain or ficin. The D antigen is unaffected by sulphydryl reagents.[46]

The LW glycoprotein is the ligand for CD11/CD18 integrins and has thus been designated as ICAM 4.[47] The transient loss of LW antigens from red cells, rarely encountered during pregnancy and in some patients with lymphoma, sarcoma, leukemia or other

types of malignancies, is deemed to be a consequence of some underlying immunological disorders.[46]

Blood group functions

During the second half of the 20th century, the work of membrane biochemists and molecular geneticists, as well as biologists, has led to multiple discoveries of functionally interesting and important membrane proteins expressed on the surface of red cells. Many authors have subdivided the membrane proteins of the erythrocyte into structural or functional groups. Thus proteins can be classified by structure, such as whether or not they are integral membrane proteins (and thus whether their N-or-C- terminals are extracellular or whether they traverse the membrane several times, as is typical for transport molecules); or proteins can be classified functionally: they may be important to the structural integrity of the cell, serve as transporters, be active as enzymes or act as receptors for a wide variety of ligands [Table 2].[48]

Blood group antigens on molecules important to membrane integrity

The majority of congenital hemolytic anemia is due to membrane defects; most are variants of hereditary spherocytosis and elliptocytosis but are not associated with unusual alteration in blood group antigens. The single exception to this is the Leach phenotype (absence of all Gerbich antigens). This phenotype is caused by total deficiency of glycophorins C and D (GPC, GPD), which are encoded by a single gene GYPC and located on glycophorin C (GPC) and glycophorin D (GPD). The phenotypes in which erythrocytes are deficient in one or more of high frequency Gerbich antigens result from different mutation and cause red cell shape abnormalities. Since GPC is an integral protein which forms complexes with membrane skeleton proteins 4.1 and the phosphoprotein P55, protein 4.1 links this complex to the spectrin/ actin network of the cytoskeleton. There is 70 to 80% reduction of GPC and GPD in hereditary elliptocytosis due to protein 4.1 deficiency.[43]

As mentioned earlier, patients with Rh_{null} phenotype have a mild hemolytic anemia with stomatocytosis and spherocytosis, pointing to a function of the Rh antigens in the maintenance of red cell structure and function.

The absence of the Kell antigens causing acanthocytosis in K_{x}-deficiency (McLoad phenotype). In the McLoad phenotype, the protein product of the X^{k} gene (Kx protein) is absent.[49] This produces acanthocytic red cells as well as a mild hemolytic anemia.[50] Erythrocytes lacking the Kx protein and antigen also have markedly weakened expression of kell blood group antigens; however, genetic defects that cause the $kell_{null}$ phenotype (with normal Kx antigen expression) do not produce red cells with shortened half-lives or abnormal morphology.[51] Perhaps most importantly however, defect in Kx gene causes neuroacanthocytosis, associated with a progressive late onset muscular dystrophy.

The other blood group antigen-bearing protein whose alteration is associated with hereditary hemolytic anemia due to membrane defects is the anion channel protein (AE1). Deficiency of band 3 in humans and animals is associated with severe hemolysis, although it is unclear whether this is due solely to lack of transport function or whether the other role of AE1 as a link between the

Table 2: Function of blood group antigens

Antigen	Gene	Gene product	Function	BG discovery
P	P	Globoside	Parvovirus B19R	Landsteiner and Levine 1927
MNSs	Mm	Glycophorin A and B	*P. falciparum* and virus R	Landsteiner and Levine 1927
LW	LW	IgSF protein (ICAM-4)	Ligand for integrins (CD11/CD18)	Levine and Stetson 1939
Rh	RH	Transport protein structure	Transported matter unknown integrity of cell metabolism and shape expression of surface Ags	Landsteiner and Wiener 1940
Lutheran	LU	IgSF protein (B-CAM)	Laminin ligand	Callender *et al* 1946
Kell	KEL	Glycoproteins	? Zn-metalloproteinases ? role in erythropoiesis	Coombs *et al* 1946
Duffy	FY	Receptor protein for	*P. vivax* and Chemokines	Cutbush *et al* 1950
Lewis	LE	Glycoconjugates	*B. pertussis* and *S. aureus* R	Grubb *et al* 1951
P, P^K, P_1	P_p, P^K	Globoside	Entral bacteria R	Levine *et al* 1951
Kidd	JK	Transporter protein	Urea transport	Allen *et al* 1951
Diego	DI	Band 3	Anion exchanger (O_2/cO_2 gas exchange)	Layrisse *et al* 1955
Cartwright	YT	GPI-linked protein in RBC integral protein in cells of the nervous system	Function unknown (Acetulcholinestrase neurotransmission)	Eaton *et al* 1956
Gerbich	GE	GlycophorinsC/D	Red cell shape and integrity	Rosenfield *et al* 1960
Colton	CO	Aquaporin-1	Water transport	Heisto *et al* 1967
In^{a}AnWJ	IN	CD44 protein	Haemophilus influenza RHyaluronic acid ligand	Badakere *et al* 1974 Telen *et al* 1993
Kx	XK	Transport protein	Neurotransporter, expression of Kell Ags, red cell membrane and muscle cell integrity	Marsh *et al* 1975
Cromer	CROM	DAF (complement)	Complement regulation. *E. coli*, Enterovirus R	Denials *et al* 1989
Knops	KN	CR1 (CD35) protein	Binding and transport of C3B/C4b coated immunocomplexes, aspecific binding of IgG	Moulds *et al* 1992
OK^a	OK	IgSF protein (CD147)	? growth factor R ? induction of extracellular metalloproteinases	

lipid bilayer and the cytoskeleton is a more important contributor to red cell instability.[52] Band 3 protein is the most abundant red cell membrane protein. It has a crucial role in O_2/CO_2 gas exchange between lungs and tissues. It serves also structural functions by interacting with skeletal proteins. Band 3 protein has only been found in erythroid cells and in intercalate cells of distal and collecting tubules of the kidney. The AE1 is the product of the Di locus (Diego). The determinants of the Di blood group system are associated with amino acid substitutions with band 3.[53] An interaction between AE1 and glycophorin A (GPA) seems to account for the Wright (Wr^a) antigen expression.[54] The deficiency of band 3 in the red cells has been considered to be lethal and no Diego-null phenotype is reported. A severely hydrophilic, anemic baby with red cells lacking band 3 has been kept alive with extreme medical intervention.[55]

Transport protein

At least three blood group systems reside on proteins that are active enzymatically: the more than 20 kell blood group antigens reside on a member of neprilysin sub-family of zinc-binding neutral endopeptidases, which include the common lymphocytic leukemia antigen (CALLA). Kx^a member of the kell antigens has also a membrane transporter structure. Kx is encoded by an X-linked gene KX, the absence of which gives rise to the rare McLoad syndrome,[56] which is characterized by deficiency or absence of the Kell antigens; by acanthocytic red blood cells; elevated serum creatinin-kinase activity; and by muscle wasting, cardiomyopathy and different neurological symptoms. The kell protein is also most closely related to the endothelin converting enzymes.[57] Cartwright is the other blood group system that resides on erythrocytes acetylcholinesterase.[58] It has been postulated but not proven that the presence of acetylcholinesterase on red cells counters diffusion of the

neurotransmitter acetylcholine away from its sites of action at neuromuscular junctions.

The Dombrock antigens reside on another glycosylphosphatidylinositol-linked protein, which is a member of the adenosine 5'- diphosphorate (ADP)-ribosyltranspherase ectoenzyme gene family, thus raising the possibility that the Dombrock protein may also function in cell adhesion.

The other three blood group systems that are known to reside on membrane transporters are – Diego system on band 3 or the anion channel protein (AE1), which is mentioned above. The Colton antigens (Co^a- Co^b and Co^3) are on a channel-forming integral protein called aquaporin-1 (AQP-1), which is a member of the family of water channel glycoproteins.[59,60] In addition to red cell, AQP-1 is strongly expressed in the kidney, enhancing the osmotically driven water transport. It also plays a significant role in water balance of the lung, cerebrospinal fluid and the eye. AQP-1 is physiologically important for urine concentration. This function is however only revealed under stress conditions.[52]

The kidd antigens Jk^a and Jk^b differ in a single amino acid substitution (Asp Asn). Jk$_3$ is a high frequency antigen present on all red cells apart of the kidd null (Jk^{a-b-}) phenotype. The kidd urea transporter is expressed in the endothelial cells of the renal vasarecta and the medulla. The kidd null phenotype is extremely rare, a part of the Polynesians, where its frequency is l:400.

The urea transport in kidd null cells is ~ 1000-fold slower than in normal cells. Kidd null phenotype generally does not cause clinical symptoms, probably because a homologous urea transporter, HUT-2, may compensate for its absence.[61]

Adhesion receptors

The adhesion proteins are members of the immunoglobulin glycoprotein super family (IgSF), characterized by repeating extracellular domains with sequence homology to the variable (V) and constant (C_1 or C_2) domain of immunoglobulins. Among proteins belonging to the IgSF, some of them are now well documented to mediate red cell adhesion: CD47 is a thrombospondin (TSP) receptor on both RBC and on other cells. Also it is found both in basement membranes as well as in a soluble form in plasma. CD47 has also been shown to bind to SIRPa and thus allow erythrocytes to be recognized as 'self' despite their lack of HLA antigens. Ligands for CD47 include integrins and thrombospondin.

CD23q or Lutheran blood group antigens function as lamini receptor on both sickle red cells as well as many epithelial cancers (B.CAM/LU); it also is a receptor for integrin. The LW protein (ICAM-4 CD24q) is capable of binding several forms of integrins commonly expressed by leukocytes and other tissues. It is the ligand for CD11/CD18 integrins and has thus been designated as ICAM4.[62] The transient loss of LW antigens from red cells, rarely encountered during pregnancy and in some patients with lymphoma, sarcoma, leukemia or other types of some malignancies, is deemed to be a consequence of some underlying immunological disorders.[62]

Other IgSF proteins include – CD108, which is on IgSF domain and known as JM-11 semaphoring K_1 (SEMA7A), may be a ligand for adhesion of activated lymphocyte CD147; or Ok^a blood group antigens, which have recently been shown to be necessary to the ability of erythrocytes to traverse the splenic bed and return to the circulation. It is present in RBCs, leukemia cells and is also widely distributed in human tissues. Apart of being a leukocyte activation factor, it has been suggested to have possible growth factor function and may be involved in tumor inavion and metastasis processes, as well as in facilitating tissue remodeling by inducing extracellular metalloproteinase (MMP).[63] It is a ligand for fibronectin, type IV collagen and laminin in other tissues.

The antigens are carried by CD44, In^a and In^b (Indian blood group system), a single pass membrane glycoprotein that is encoded by the CD44 gene on chromosome 11 at position p13. It is a leukocyte homing receptor and adhesion molecule. The protein is widespread and found in a large variety of tissues. It is found most commonly in Iranians and Arabs.[64] The RBC deficient in CD44 has only been described in an individual who had an unusual presentation of congenital dyserythropoetic anemia (CDA). CD44 binds to hyaluronan, collagen, fibronectin and lamini. CD44 also cooperates with VLA-4 as a fibronectin receptor.[65]

CD99 is the product of Mic2 gene, known to mediate RBC-lymphocyte interaction and T cell rosette formation.[65]

CD58 on red cells also adheres to CD2 (is a ligand for CD2) on activation of T cells, resulting in rosette formation. It is known as lymphocyte-associated antigen-3 (LFA-3).[65]

Other blood group antigens having a receptor function

The Fy protein is a transmembrane glycoprotein of 35-43 KD^a consisting of a glycosylated amino-terminal region, which protrudes from the membrane. The protein is predicted to have seven transmembrane spanning domains. In 1993, it was realized that Fy was the erythrocyte chemokine receptor that could bind IL-8 and MCP-1, and the cloning of the gene confirmed that it belongs to the family of chemokine receptors.[66] Because of this association, Fy protein was named DARC (Duffy antigen receptor for chemokines). Chemokine receptors have been principally found on lymphocytes, where they couple to G-proteins and activate intracellular signaling pathways that regulate cell migration into tissues.[67] The importance of Fy as a receptor for the malarial parasite *P. vivax* is well established, but its biological role as a chemokine receptor on RBCs, endothelial cells and brain is not yet clear. Investigators have hypothesized that Fy may act as a scavenger or sink for excess chemokine release into the circulation.[68] If the function of Fy on RBCs is to scavenge excess chemokine, this might predict that Fy[a-b-] individuals would be more susceptible to septic shock or to cardiac damage following infarction.[68] Because African Americans have higher rates of renal allograft rejection and Fy has been shown to be up regulated in the kidney during renal injury[69] and graft rejection,[70,71] chemokines on erythrocytes have also been suggested to predispose African American men to greater incidence and mortality of prostate cancer.[72]

Blood group antigens with complement-related function

At least three well investigated erythrocyte membrane proteins are involved in the regulation of complement and the clearance of immune complexes. The C3b/C4b receptor, also known as complement receptor type 1 (CR1), is the Knops/McCoy blood group antigen[73] and is believed to be important in immune adherence.[74] Recently, the presence of a naturally occurring low expression polymorphism of CR1 has been associated with the occurrence of more severe malaria.[75]

The proteins decay accelerating factor (DAF, CD55) and membrane inhibitor of reactive lysis proteins (MIRL-CD59) were identified later, largely due to investigation of the complement regulatory defect of red cells in paroxysmal nocturnal hemoglobinuria (PNH).[76]

Both CD55 and CD59 are attached to the membrane by glycosylphosphatidylinositol anchors and are thus absent from PNH red cells, in which the synthesis of such anchor is defective.[76] CD 55 carrier the Cromer blood group system, whose null phenotype is associated with a subclinical defect in membrane complement regulation. No blood group antigen has thus been identified on CD59.

Receptors on viruses and bacteria

Many bacteria strains bind to a galactose-α_1-motif shared by the blood group antigens P, P_k and P_1 and are responsible for enteral and upper urinary tract infections. Glycoconjugates carrying Lewis^a specificity bind B pertussis and staphylococcus aureus toxins, the later having been implicated in the sudden infant death syndrome.[77] Blood group glycoproteins with Lewis^b specificity bind Helicobacter pylori, which is known to cause peptic ulcers, chronic gastritis and gastric carcinoma.[78]

The receptor of parvovirus B_{19} is the P glycolipid (globoside) on the surface of erythroid progenitors. The viral infection mostly causes benign anemia, but in immunosuppressed patients it results in aplastic anemia. Individuals with p phenotype are resistant to

the infection by parvovirus B$_{19}$.[79] One of the regulatory proteins of the complement cascade, DAF functions as receptor for the DR. family of adhesions from E. coli strains, which are causative agents of lower urinary tract infection. DR. antigens are also receptors for several serotypes of echoviruses and coxachi viruses that are implicated in different febrile illnesses, including aseptic meningitis.[80]

Acknowledgment

References

1. Landsteiner K. Zur Kenntnis der anti-fermentativen lytrschen und agglutinierenden wirkungen des Bluteerums und der lymphe. Zentralbi Bakteriol 1900;27:357-66.

2. Landsteiner K. Uber Agglutination serscheinungen normalen menschichen Blutes. Wien Klin Wschr 1901;14:1132.

3. Dungern EV, Hirszfield L. Uber gruppenspezifizche strukturen des Blutes III. Z Immun Forsch 1911;8:526-62.

4. Race RR, Sanger S. Blood Group in man. 6th ed. Blackwell: Oxford; 1975.

5. Landsteiner K, Levine P. A new agglutinable factor differentiating individual human bloods. Proc Soc Exp Biol N Y 1927;24:600-2.

6. Landsteiner K, Wiener AS. Studies on an agglulinagen (Rh) in human blood reacting with anti-rhesus sera and with human isoantibodies. J Exp Med 1941;74:309.

7. Levine P, Stetson RE. Unusual case of intra group agglutination. J Am Med Assoc 1939;113:126-7.

8. Diamond LK, Denton RL. Rh agglutination in various media with particular reference to the value of albumin. J Lab Clin Med 1945;30:821-30.

9. Mollison PL. Blood transfusion in clinical medicine. 6th ed. Blackwell: Oxford; 1979.

10. Coombs RR, Mourant AE, Race RR. Detection of weak and "incomplete" Rho agglutinins a new test. Lancet 1945;2:15.

11. Pourazar A. Ph.D thesis. Changes in blood group ABH and I antigens in leukemia and other hematological malignancy patients. ICMR: India; 1984. p. 124.

12. Bhende YM, Deshpande CK, Bhatia HM, Sanger R, Race RR, Morgan WT, et al. A "new" blood group character related to the ABO system. Lancet 1951;1:903-4.

13. Levine P, Robinson E, Celano M, Briggs O, Falkinburg L. Gene interaction resulting in suppression of blood group substance B. Blood 1995;10:1100-8.

14. Watkins WM, Morgan WT. Possible genetical pathways for the biosynthesis of blood group mucopolysaccharides. Vox Sang 1959;4:97-119.

15. Bhatia HM, Sathe MS. Incidence of "Mumbai" (Oh) phenotype and weaker variants of A and B antigen in Mumbai (India). Vox Sang 1974;27:524-32.

16. Joshi SR, Pourazar A, Clark VA, Ala FA. Para-Bambay phenotype with altered I-I blood group in an irani donor. Transfusion Today 2000.

17. Pourazar A, Joshi SR, Clarke VA, Ala F. Another cases of parabombay phenotype in an Irani donor. Arch Iranian Med 2004;7:284-6.

18. Grubb R. Observation on the human group system Lewis. Acta Path Microbiol Scand 1951;28:61.

19. Sneath JS, Sneath PH. Transformation of the Lewis groups of human red cell. Nature 1955;176:172.

20. Cutbush M, Giblett ER, Mollison PL. Demonstration of the phenotype Le$^{(a+,b+)}$ in infants and in adult. Br J Haematol 1956;2:210-20.

21. Kissmeyer-Nielsen F. Irregular blood group antibodies in 200,000 individuals. Scand J Haematol 1965;2:331-42.

22. Mollison PL, Engelfrient CP, Contreas M. Blood transfusion in clinical medicine. 10th ed. Blackwell: 1997. p. 138-9.

23. Brown KE, Hibbs JR, Gallinella G, Anderson SM, Lehman ED, McCarthy P, et al. Resistance to parvovirus 1319 infection due to lack of virus receptor (erythrocyte P antigen). N Engl J Med 1994;330:1192-6.

24. Dunstan RA, Simpson MB. Heterogeneous distribution of antigens on human platelets demonstrated by fluorescence flow cytometry. Br J Hematol 1985;61:603-9.

25. Cameron GL, Stavely JM. Blood group P substance in hydatid cyst fluids. Nature 1975;179:147-8.

26. Fellous M, Gerbal A, Tessier C, Frezal J, Dausset J, Salmon C. Studies on the biosynthetic pathway of human P erythrocyte antigens using somatic cells in culture. Vox Sang 1974;26:518-36.

27. Burnie K. Ii antigens and antibodies. Can J Med Technol 1973;55:5-7.

28. Testa U, Rochant H, Henri A, Titeux M, Ton That H, Vainchenker W. Charge in i antigen expression of erythrocytes during in vivo aging. Rev Fr Transfus Immunohematol 1981;24:299-305.

29. Joshi SR, Bahtia HM. A new red cell phenotype I-i:red cell lacking in leukemia and other hematological malignancy patients. ICMR: India; 1984. p. 124.

30. Page PL, Langevin S, Petersen RA, Kruskall MS. Reduced association between the Ii blood group and congenital cataracts in white patients. Am J Clin Pathol 1987;87:101-2.

31. Watkins WM. Biochemistry of genetics of the ABO, Lewis, and P blood group systems. Adv Hum Genet 1980;10:1-136.

32. Hakomori S. Blood group ABH and Ii antigens of human erythrocytes: Chemistry, polymorphisms and developmental changes. Semin Hematol 1981;18:39-62.

33. Mark E, Brecher, et al. Technical manual. 50th anniversary AABB ed. 14th ed. 1953-2003. 2003. p. 272-96.

34. Avent ND, Reid ME. The Rh blood group system: A review blood 2000;95:375-87.

35. Shah D, Salvia V. The rhesus factor. 1st ed.1998. p. 9-25.

36. Wagner FF, Flegal WA. Review: The molecular basis of the Rh blood group phenotypes. Immunohaematology 2004;20:23-36.

37. Hughes-Jones NC, Gardner B, Lincoln PJ. Observations of the number of available c, D, e and E antigen sites on red cells. Vox Sang 1971;21:210-6.

38. Masouredis SP, Sudara EJ, Mahan L, Victoria EJ. Antigen site densities and ultra-structural distribution pattern of red cell Rh antigens. Transfusion 1976;16:94-106.

39. Josh SR. A perspective on Blood Groups published by pernatology committee. Federation of obstetric and Gynaecological Societies of India: 1998. p. 9-24.

40. Hung CH, Liu PZ, Cheng JG. Molecular biology and genetics of the Rh blood group system. Semin Hematol 2000;37:150-65.

41. Hadley AG. Laboratory assays for predicting the severity of haemolytic disease of the fetus and newborn. Transpl Immunol 2002;10:191-8.

42. Agra P, Cartron JP. Biochemistry and molecular genetics of Rh antigens in Baillieres Clin Hematol 1991;4:493-891.

43. Westhoff CM, Reid ME. Review: The Kell, Duffy and Kidd blood group systems. Immunohematology 2004;20:37-49.

44. Homayouni V, Pourazar A, Rezaei A. Comparison evaluation of infant fetal erythrocytes with haemolytic Disease of Newborn (HDN), in maternal blood using flow cytometric methods. Isfahan University of Medical Science: MSc thesis; 2005.

45. Lomas CG, Tippet P. Use of enzymes in distinguishing anti-

LW^a and LW^{ab} from anti-D. Med Lab Sci 1985;42:88-9.

46. Danieles G. Human blood group. Oxford: Blackwell; 1995.

47. Bailly P, Tontii E, Hermand P, Cartron JP, Gahmberg CG. The red cell LW Blood group protein is an intercellular adhesion molecule which binds to CD11/CD18 leucocyte integrins. Eur J Immunol 1995;25:3316-20.

48. Garratty G, Telen MJ, Petz DL. Red cell antigens as functional molecules and obstacles to transfusion. Hematology Am Soc Hematol Educ Program 2002:445-62.

49. Khamlichi S, Bailly P, Blanchard D, Goossens D, Gartron JP, Bertrand O. Purification and partial charaterization of the erythrocyte Kx protein deficient in McLeod patients. Eur J Biochem 1995;228:931-4.

50. Danek A, Rubio JP, Rampoldi L, Ho M, Dobson-Stone C, Tison F, et al. McLeod neuroacanthocytosis: Genotype and phenotype. Ann Neurol 2001;50:755-64.

51. Lee S, Lin M, Mele A, Cao Y, Farmar J, Russo D, et al. Proteolytic processing of big endothelin-3 by the kell blood group protein. Blood 1999;94:1440-50.

52. Roudier N, Verbavatz JM, Maurel C, Ripoche P, Tacnet F. Evidence for the presence of aquaporin-3 in human red blood cells. J Biol Chem 1998;237:8407-12.

53. Zelinski T. Erythrocyte band 3 antigens and the Diego blood group system. Transfus Med Rev 1998;12:36-45.

54. Telen MJ. Erythrocyte blood group antigens: Not so simple after all. Blood 1995;85:299-306.

55. Daniels G. Functional aspects of red cell antigens. Blood Rev 1999;13:14-35.

56. Daniels GL, Weinauer F, Stone C, Ho M, Green CA, Jahn-Jochem H, et al. A combination of effects of rare genotypes at the XK and KEL blood group loci results in the absence of Kell system antigens from the red blood cells. Blood 1996;88:4045-50.

57. Lee S, Russo DC, Reiner AP, Lee JH, Sy MY, Telen MJ, et al. Molecular defects underlying the Kell null phenotype. J Biol Chem 2001;276:27281-9.

58. Yu LC, Twu YC, Chang CY, Lin M. Molecular basis of the Kell-null phenotype: A mutation at the splice site of human KEL gene abolishes the expression of Kell blood group antigens. J Biol Chem 2001;276:10247-52.

59. Smith BL, Agre P. Erythrocyte M-28000 transmembrane protein exists as a multi-subunit oligomer similar to channel proteins. J Biol Chem 1991;266:6407-15.

60. Agre P, Smith BL, Preston GM. ABH and Colton blood group antigens on aquaporin-1, the human red cell water channel protein. Transfus Clin Biol 1995;2:303-8.

61. Olives B, Martial S, Mattie MG, Matassi G, Rousselet G, Ripoche P, et al. Molecular characterization of a new kidney urea transporter in the human kidney. FEBS Lett 1996;386:156-60.

62. Bailly P, Tontti E, Hermand P, Cartgron JP, Gahmberg CG. The red cell LW blood group protein is an intercellular adhesion molecule which binds to CD11/CD18 leucocyte integrins. Eur J Immunol 1995;25:3316-20.

63. Biswas C, Zhang Y, DeCastro R, Guo H, Nakamura T, Kataoka H, et al. The human tumor cell-derived collagenase stimulating factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. Cancer Res 1995;55:434-9.

64. Telen MJ, Green AM. The Inab phenotype: Characterization of the membrane protein and complement regulatory defect. Blood 1989;74:437-41.

65. Springer TA. Adhesion receptors of the immune system. Nature 1990;346:425-34.

66. Chaudhuri A, Polyakova J, Zbrzenzna V, Williams K, Gulati S, Pogo AO. Cloning of glycoprotein D cDNA, which encodes the major subunit of the Duffy blood group system and the receptor for the Plasmodium vivax malaria parasite. Proc Natl Acad Sci USA 1993;90:10793-7.

67. Tilg H, Shapiro L, Atkins MB, Dinarello CA, Mier JW. Induction of circulating and erythrocyte-bound IL-8 by IL-2 immunotherapy and suppression of its in vitro production by IL-1 receptor antagonist and soluble tumor necrosis factor receptor (p75) chimera. J Immunol 1993;151:3299-307.

68. de Winter RJ, Manen A, de Jong YP, Adams R, van Deventer SJ, Lie KI. Interleukin 8 released after acute myocardial infarction is mainly bound to erythrocytes. Heart 1997;78:598-602.

69. Chaudhuri A, Nielsen S, Elkjaer ML, Zbrzezna V, Fang F, Pogo AO. Detection of Duffy antigen in the plasma membranes and cabeolae of vascular endothelial and epithelial cells of non erythroid organs. Blood 1997;89:701-12.

70. Liu XH, Hadley TJ, Xu L, Peiper SC, Ray PE. Up-regulation of Duffy antigen receptor expression in children with renal disease. Kidney Int 1999;55:1491-500.

71. Segerer S, Bohmig GA, Exner M, Colin Y, Cartron JP, Kerjaschki D, et al. When renal allograft turn DARC. Transplantation 2003;75:1030-4.

72. Lentsch AB. The Duffy antigen/receptor for chemokines (DARC) and prostate cancer. A role as clear as black and white? FASEB J 2002;16:1093-5.

73. Rao N, Ferguson DJ, Lee SF, Telen MJ. Identification of human erythrocyte blood group antigens on the C3b/C4b receptor. J Immunol 1991;146:3502-7.

74. Tas SW, Klickstein LB, Barbashov SF, Nicholson-Weller A. C1q and C4b bind simultaneusly to CR1 and additively support erythrocyte adhesion. J Immunol 1999;163:5056-63.

75. Nagayasu E, Ito M, Akaki M, Nakano Y, Kimura M, Looareesuwan S, et al. CR1 density polymorphism on erythrocytes of falciparum malaria patients in Thailand. Am J Trop Med Hyg 2001;64:1-5.

76. Telen MJ. Glycosyl phosphatidylinositol-linked blood group antigens and paroxysmal nocturnal hemoglobinuria. Transfus Clin Biol 1995;2:277-301.

77. Saadi A, Weir DM, Poxton IR, Stewart J, Essery SD, Blackwell CC, et al. Isolation of an adhesin from Staphylococcus aureus that binds Lewis a blood group antigen and its relevance to sudden infant death syndrome. FEMS Immunol Med Microbiol 1994;8:315-20.

78. Boren T, Falk P, Roth KA, Larson G, Normare S. Attachment of Helicobacter pylori to gastric epithelium mediated by blood group antigen. Science 1993;262:1892-5.

79. Brown KE, Anderson SM, Young NS. Erythrocyte P antigen: Cellular receptor for B19 parvovirus. Science 1993;262:114-7.

80. Cartron JP, Bailly P, Le Van Kim C, Cherif-Zahar B, Matassi G, Bertrand O, et al. Insights into the structure and function of membrane polypeptides carrying blood group antigens. Vox Sang 1998;74:29-64.