Red cell antigens: Structure and function

Abbasali Pourazar

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 and A2 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.[6]

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
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-A/B (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 groups.[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\(^a\)\(^{k+}\) if they are nonsecretors (se se), but the phenotype Le\(^a\)\(^{k−}\) if they are secretors – Grubb 1951, Ceppellini 1955.[17] Cord red cells do not react with anti-Le\(^a\) and are not agglutinated by anti-Le. 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\(^1\) simply implies P 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\(^2\) 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 scolices 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\(^2\) were found on platelets and their distribution was also heterogeneous.[24] P\(^1\) and P\(^2\) are present on lymphocytes and fibroblasts. P\(^4\) 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 to 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](https://www.medknow.com)
undetectable I antigen levels.[27]

Antigens of the li 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.[24] Li-deficient phenotypes have been described, and dominant inheritance pattern was suggested. The Li 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 P1 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 li 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: H1 and H2 are not branched whereas H3 and H4 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−, B+, B−, H+, H− with i antigen. Larger concentrations of branched ABH antigens are found on adult red cells: A+, A−, B+, B−, H+, H−, H+, I− 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, Le (a− b+) will have a higher level than one who is A, 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-P1) 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-IIH, which requires the presence of both H and I antigens to react. There are many other interaction antibodies, such as anti-IP1 and anti-A, Lea.[33]

Figure 2: Schematic representation of the red cell membrane showing antigen-bearing glycosylation of proteins and lipids GPI = glycosphosphatidylinositol.
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 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- subjects in the RBC membrane, the Rh proteins form a complex

| 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+ Gene complex | Short notation | RhD- Gene complex | Short notation |
|-------------------|----------------|-------------------|----------------|
| CDe               | R<sup>1</sup>  | cde               | r              |
| cDE               | R<sup>2</sup>  | Cde               | r              |
| CDe               | R<sup>3</sup>  | cde               | r              |
| CDE               | R<sup>4</sup>  | Cde               | r              |

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 immunogens. 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, Hughes-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 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, Rh6), Ce (also known as rh, Rh7) CE (Rh22), cE (Rh27). Of these, ce has its variant called ce<sup>+</sup> (also called V, hr<sup>+</sup> or Rh10), 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<sup>+</sup>t) or Cde/cDE (r<sup>-</sup>t). However, the red cells reacting with anti-<i>c</i>- (f) or anti-<i>C</i>- and not with anti-<i>Ce</i>- (Rh) or anti-cE would confirm an actual genotype of that person to be Cde/cde.

Antigen G (rh<sup>C</sup>, Rh:12) is found on almost all D<sup>-</sup> or C<sup>-</sup> 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<sup>+</sup>. Antigens C<sup>+</sup> (Rh8), C<sup>-</sup> (Rh9) and MAR (Rh51) represent alleles at the C-c locus of which C<sup>+</sup> and C<sup>-</sup> 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<sup>+</sup> (Rh24) 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<sup>+</sup> (Rh:11) is defined by specific anti-E<sup>+</sup>. The hr<sup>-</sup> (Rh:19) and hr<sup>+</sup> (Rh:31) represent partial <i>e</i> antigens usually found in the population of African origin; V (ce<sup>+</sup>, [Rh:10]) and VS (e<sup>+</sup>, [Rh:20]) and R<sup>-</sup> are further antigens encountered among the Black population.[39]

The phenotype described as Rh<sub>null</sub> may be produced by at least two different genetic mechanisms. In the more common regulator

**Figure 3:** Biosynthesis of antigens of the ABO, Li, P and Lewis systems (Watkin W.M. 1980)
type of Rh\textsubscript{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\textsubscript{null} show overall depression of their Rh antigens known as Rh\textsubscript{null}null. The other form of Rh\textsubscript{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\textsubscript{null} is considerably rarer than the regulator type. Parents and offspring of this type of Rh\textsubscript{null} are obligate heterozygotes for the amorph.\textsuperscript{[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.\textsuperscript{[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\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[35]}

The antigens are denatured by sulphhydryl reagents such as DTT but are unaffected by papain or ficin. The D antigen is unaffected by sulphhydr reagents.\textsuperscript{[46]}

The LW glycoprotein is the ligand for CD11/CD18 integrins and has thus been designated as ICAM 4.\textsuperscript{[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.\textsuperscript{[48]}

**Blood group functions**

During the second half of the 20\textsuperscript{th} 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].\textsuperscript{[49]}

**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 glycoporphins C and D (GPC, GPD), which are encoded by a single gene GYPC and located on glycoporphin C (GPC) and glycoporphin 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.\textsuperscript{[43]}

As mentioned earlier, patients with Rh\textsubscript{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\textsubscript{x} deficiency (McLoad phenotype). In the McLoad phenotype, the protein product of the X\textsuperscript{k} gene (Kx protein) is absent.\textsuperscript{[49]} This produces acanthocytic red cells as well as a mild hemolytic anemia.\textsuperscript{[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\textsubscript{null} phenotype (with normal Kx antigen expression) do not produce red cells with shortened half-lives or abnormal morphology.\textsuperscript{[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    | Mn   | 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 |
| Lutherian | LU   | IgSF protein (B-CAM) | Laminin ligand | Callender et al 1946 |
| Kell    | KEL  | Glycoproteins | ? Zn-metalloproteinases | 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 |
| Kidd    | JK   | Transporter protein | Enterobacteria R | Levine et al 1951 |
| Diego   | DI   | Band 3 | Urea transport | Allen et al 1951 |
| Cartwright | YT   | GPI-linked protein in RBC integral protein in cells of the nervous system | Anion exchanger (O2/CO2 gas exchange) | Layrisse et al 1955 |
| Gerbich | GE   | Glycoconins G/D | Function unknown (Acetylcholinesterase neurotransmission) | Eaton et al 1956 |
| Colton  | CO   | Aquaporin-1 | Red cell shape and integrity | Rosenfield et al 1960 |
| IN      | IN   | CD44 protein | Water transport | Heisto et al 1967 |
| Kx      | XK   | Transport protein | Haemophilus influenza | Badakere et al 1974 |
| Cromer  | CROM | DAF (complement) | RHyaluronic acid ligand | Telen et al 1993 |
| Knops   | KN   | CR1 (CD35) protein | Neurotransporter, expression of Kell Ags, red cell membrane and muscle cell integrity | Marsh et al 1975 |
| OK+     | OK   | IgSF protein (CD147) | Complement regulation. E. coli, Enterovirus R | Denils et al 1989 |
|         |      |              | Binding and transport of G6PC4-b coated immunocomplexes, aspecific binding of IgG | Moulds et al 1992 |

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 O2/CO2 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+) antigen expression.[14] 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 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 McLeod 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'-phosphoribosyltransferase 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- Co and Co) 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+ and Jk- differ in a single amino acid substitution (Asp Asn). Jk is a high frequency antigen present on all red cells apart of the Kidd null (Jk-+) 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 1:400.

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\textsubscript{\textalpha}, C\textsubscript{\textbeta}) 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 SIRP\alpha 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.\textsuperscript{42} 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.\textsuperscript{42}

Other IgSF proteins include – CD108, which is on IgSF domain and known as JM-11 semaphoring K\textsubscript{\textalpha} (SEMA7A), may be a ligand for adhesion of activated lymphocyte CD147; or Ok\textsuperscript{\textalpha} 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 invasion and metastasis processes, as well as in facilitating tissue remodeling by inducing extracellular metalloproteinase (MMP).\textsuperscript{43} It is a ligand for fibronectin, type IV collagen and laminin in other tissues.

The antigens are carried by CD44, In\textsuperscript{\textgamma} and In\textsuperscript{\textdelta} (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.\textsuperscript{44} The RBC deficient in CD44 has only been described in an individual who had an unusual presentation of congenital dyserythropoietic anemia (CPDA). CD44 binds to hyaluronan, collagen, fibronectin and lamin. CD44 also cooperates with VLA-4 as a fibronectin receptor.\textsuperscript{45}

CD99 is the product of Mic2 gene, known to mediate RBC-lymphocyte interaction and T cell rosette formation.\textsuperscript{46}

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).\textsuperscript{46}

Other blood group antigens having a receptor function

The Fy protein is a transmembrane glycoprotein of 35-43 KD\textsuperscript{\textalpha} 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.\textsuperscript{46} 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.\textsuperscript{47} The importance of Fy as a receptor for the malarial parasite \textit{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.\textsuperscript{48} If the function of Fy on RBCs is to scavenge excess chemokine, this might predict that Fy\textsuperscript{a\textbeta\textgamma\textdelta} individuals would be more susceptible to septic shock or to cardiac damage following infarction.\textsuperscript{48} 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\textsuperscript{49} and graft rejection,\textsuperscript{50,51} chemokines on erythrocytes have also been suggested to predispose African American men to greater incidence and mortality of prostate cancer.\textsuperscript{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 CD3/C4b receptor, also known as complement receptor type 1 (CR1), is the Knops/McCoy blood group antigen and is believed to be important in immune adherence.\textsuperscript{46} Recently, the presence of a naturally occurring low expression polymorphism of CR1 has been associated with the occurrence of more severe malaria.\textsuperscript{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).\textsuperscript{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.\textsuperscript{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-\textalpha\textgamma-motif shared by the blood group antigens P, P\textgamma, and P\textdelta and are responsible for enteral and upper urinary tract infections. Glycoconjugates carrying Lewis\textalpha specificity bind B pertussis and staphylococcus aureus toxins, the later having been implicated in the sudden infant death syndrome.\textsuperscript{77} Blood group glycoproteins with Lewis\textbeta specificity bind Helicobacter pylori, which is known to cause peptic ulcers, chronic gastritis and gastric carcinoma.\textsuperscript{78}

The receptor of parvovirus B\textsubscript{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 B19.\textsuperscript{[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 coxsackieviruses that are implicated in different febrile illnesses, including aseptic meningitis.\textsuperscript{[80]}

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