

The Structure and Function of the Molecules That Carry Human Red Blood Cell and Platelet Antigens

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A number of molecules on the surface of red blood cells (RBCs) and platelets express antigenic activity. Various biochemical and molecular approaches have been used to determine the structure and possible function that these molecules have for their respective cell types. The existence of variant molecules and null phenotypes and the immunological response to these antigens have aided in the analysis of the structure and function relationships of these molecules. A comparison of the sequence to moieties of known function and the presence of functional domains for many of the molecules allows for a prediction of their function. The proposed function of the molecules that express RBC and platelet antigens includes membrane structure, transporter or channel formation, receptor/ligand signaling or adhesion, en-

zyme activity, and glycocalyx formation. However, the function of some of these molecules is not known, and many of the variant antigens do not show an obvious functional difference. For unknown reasons, some of these molecules are exceptionally polymorphic and the elucidation of the precise role that these polymorphisms play in structure and function is hindered by limitations in the *in vitro* and *ex vivo* analyses and access to precursor cell types. The objective of this review is to define the structure and function of those molecules that express RBC and platelet antigens and the significance, if any, that polymorphisms have for these molecules.

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WE HAVE LEARNED from serological and biochemical studies of the immune response to transfusions that cellular blood products have on their surface a number of molecules that are antigenic in other humans. They are antigenic in the sense that some individuals who are transfused or become pregnant (transplacental exchange of blood) will develop antibodies to “foreign” epitopes expressed on these molecules. These antibodies identify phenotypic variants of the molecules that can be characterized by distinct and inheritable alleles. By the 1950s, the pre- and post-transfusion serological analyses that was part of compatibility testing, now known as the field of immunohematology, began to witness a dramatic rise in the identification of red blood cell (RBC) antigens.^{1,2} At the time, platelet antigens were more difficult to characterize because platelets required more sophisticated techniques to avoid spurious results. The first human platelet antigen (HPA) system, Zw/PI^A or HPA-1, was identified in 1959³ and there followed a hiatus of more than 20 years before the next system was characterized.⁴ To date, there are 29 RBC and 5 platelet antigen systems established. More than 270 antigens and 650 alleles have been identified for the RBC systems alone. The purpose of the structural diversity for the many molecules expressed on the surface of blood cells is not entirely known, but the antibodies that develop as a result of transfusion or pregnancy and the xenoantibodies produced by the deliberate immunization of mice or rabbits have

proved useful for the study of the structure and function of these molecules.

Antigens result from the expression of genetic changes that range from single nucleotide substitutions to intra- or intergenic conversions, inversions, deletions, or other rearrangements. A RBC or platelet antigen system may consist of 2 alleles (expressed as 2 codominant antigens) that differ by one nonsynonymous nucleotide substitution or be comprised of a group of highly homologous genes each with a few to several dozen allelic variants. The genome of a person contains only 2 alleles at any given autosomal locus, and it is impressive that many of the alleles have been characterized at the molecular level. Together with the biochemical analysis of various phenotypes, we have a good understanding of the orientation and relationship of these molecules to other plasma membrane and cytoplasmic constituents. The present challenge is to determine the function of those molecules that

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express such antigens and the effect, if any, that RBC and platelet genetic polymorphisms have on their function.

This review will focus on the structural aspects of the molecules that express RBC and platelet antigens, the function or possible function of these molecules, and the potential biological significance of their polymorphisms.

HISTORICAL PERSPECTIVES

Human Polyclonal Antibodies

Most of the knowledge for RBC and platelet antigenic variation comes from the serological analyses of both related and unrelated individuals. Some of the best examples come from the Rh blood group system. The D antigen is a highly immunogenic nonglycosylated transmembrane protein that was first identified using serum from postpartum women whose infants had hemolytic disease of the newborn and from ABO-compatible blood transfusion recipients.^{5,6}

Antibodies found in the sera of D-negative immunized individuals are directed to multiple epitopes on the Rh protein.⁷⁻⁹ The antibodies are "polyclonal" in the sense that the B lymphocytes recruited to produce the antibodies arise from different lineages. They differ by the immunoglobulin heavy- and light-chain variable region genes used to define their antigen specificity. The antibodies are directed to a finite number of epitopes that are unique to the D antigen, which is limited by the structural similarities of the *RHCE* gene product. However, some of the antibodies from the different B lymphocytes may be directed to similar epitopes. On the other hand, gene conversion and exon/intron recombinations between *RHD* and *RHCE* has led to the expression of altered or "variant" forms of the D protein (eg, category D^{VI}), which can result in the development of anti-D in the D^{VI}-positive individual.¹⁰ These antibodies are directed toward the epitope(s) on the wild-type D molecule that are missing on the variant protein.

It was realized quickly that many of the emerging human polyclonal antibodies were reliable reagents to phenotype cells using agglutination or, as in the case of platelets, using other immunoglobulin-based techniques like immunoblotting and immunoprecipitation. It is because simple agglutination is not a reliable technique to phenotype platelets that these techniques were necessary.

Platelets express a significant amount of immunoglobulin (Ig) G on their surface and can mimic agglutination by aggregating under less than ideal conditions. Unexpected reactions are also evident among those RBC antigen systems with large numbers of allelic variants like Rh and MNS.^{11,12} It was the study of the different serological reaction patterns for these variant molecules using an array of antibodies that helped us understand the structure of these molecules. For example, the noncovalent association of RBC membrane-associated band 3 and glycophorin (GYP)A was determined by the analysis of human anti-Wr^b versus anti-Wr^a antibodies.^{13,14}

Monoclonal Antibodies

Monoclonal antibodies can be derived either from Epstein-Barr virus transformed human B lymphocytes or from splenic lymphocytes of deliberately immunized mice. These specific, high-affinity antibodies, obtained using a technique first developed by Kohler and Milstein,¹⁵ can be used as hemagglutinins or in some instances to immunoprecipitate or immunoblot membrane proteins separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Murine antibodies have been produced with specificities to the ABO, MNS, Kell, Duffy, and Dombrock blood group antigens. However, murine antibodies often recognize xenoepitopes present on the molecules regardless of any blood group antigen. Overall, monoclonal antibodies are useful reagents to (1) isolate the molecules that express blood group antigens,¹⁶ (2) confirm the expression of extracellular motifs,¹⁷ and (3) identify functional domains.^{18,19} However, one must exercise caution with the use of these antibodies because the presence of a particular motif does not always imply a specific allele; some motifs can arise from different genetic mechanisms. For example, the low-incidence Rh antigen, FPTT, is expressed by DFR and by Ro^{HAR} RBCs and yet the molecular changes for these 2 D-variant alleles are different.^{20,21}

Biochemical Studies

During the 1970s, the biochemical approach to study the structure of plasma membranes led to the identification of the major proteins and glyco-conjugates on the RBC membrane. This was largely because of the fact that RBC membranes or "RBC ghosts" could be isolated with relative ease.²² The

biochemical structures of the A and B carbohydrate antigens were the first to be characterized because molecules expressing these antigenic determinants were readily available in soluble form.^{23,24} Molecules present in high copy number on RBCs (eg, band 3) were characterized largely because of their abundant nature. This fact also enabled the isolation of the molecule to sufficient purity for direct amino acid sequencing. However, the characterization and isolation of membrane proteins in low-copy number required immunoprecipitation combined with SDS-PAGE, a technique that proved particularly useful for platelet membrane glycoproteins. Furthermore, SDS-PAGE using nonreduced and reduced conditions revealed covalent intra- and intermolecular disulfide bonds.²⁵ This work allowed researchers to determine the membrane orientation and interactions between molecules when combined with isotopic labeling and mild enzymatic digestion of intact cells. To this end, a unique plasma membrane linkage was identified using phospholipases and showed that some proteins are attached to the membrane by a glycosylphosphatidylinositol (GPI) anchor.²⁶

Null Phenotypes

Another major advancement in our understanding of the function of molecules that express blood group antigens came from the analysis of null phenotypes. When transfused, individuals with a null phenotype can become alloimmunized and the corresponding antibodies produced are useful particularly for the biochemical analysis of the molecule. The analysis of the Rh null phenotype led to the hypothesis that Rh proteins exist within the plasma membrane as a complex that includes Rh50.²⁷ Immunoprecipitation studies with antibodies from Kell null individuals led to the discovery of the relationship between Kell and Xk glycoproteins.^{28,29} Also, some null phenotypes are associated with observable clinical abnormalities (eg, altered shape and shortened survival, abnormal function, or are associated with an inheritable RBC disorder).³⁰⁻³⁴ Furthermore, antibodies from transfused null phenotypes may interfere with the *in vitro* function of the molecule as has been shown using the antibodies found in immunized Glanzmann's thrombasthenic patients.³⁵

Molecular Analyses

Molecular sequence analysis is a tool that provides insight into the proposed structure or function of the genes that express blood group antigens.³⁶ Computational analyses can compare a putative sequence with the genes whose sequence and function are known. This work provides an added dimension to the characterization of most blood group antigens and their variants. Initially, gene cloning required the purification of the expressed molecule using, for example, highly specific antibodies followed by amino acid sequencing. Current techniques include the use complementary DNA (cDNA) expression libraries and specific antibodies to isolate candidate genes provided that the manipulation does not alter the target epitope. Variations with the use of cDNA libraries include screening libraries with degenerated synthetic probes devised from short amino acid sequences. Today, techniques used for cloning and sequencing are considered routine in most molecular biology laboratories. The cDNA sequence and knowledge of the exon/intron boundaries of the gene provides sufficient information to allow the cloning of variant alleles using genomic DNA and the polymerase chain reaction. For example, genomic DNA can be used to determine the molecular changes for *RHD* variant alleles even though the entire genomic sequence is not yet known. However, it may not be possible to characterize all unusual variants if another gene is involved in its expression (eg, RhAG is required for the expression of Rh) or if the entire sequence of the gene is not known.

The nucleic acid sequence only provides the information for the primary amino acid structure. On the other hand, crystal structure of the protein provides critical information that can predict the effects of amino acid substitutions. Unfortunately, the crystallographic coordinates for the molecules that express RBC and platelet antigens are not yet available. It is the computational analysis of the deduced amino acid sequence that allows the prediction of the tertiary structure, functional domains, and insight into the possible role of the molecule. First, hydropathy plots identify potential transmembrane domains and, along with the identification of potential N-linked and O-linked glycosylation sites, can predict the orientation of the molecule in the plasma membrane.³⁷ Second, the

sequence homology can be compared with those sequences already in databases such as GenBank. Overall, sequence homology or regions of homology to proteins of known structure and function (especially those with crystallographic co-ordinates) identifies a potential role for the molecule. Beyond the computational analysis, once the cDNA clone has been obtained, site-directed mutagenesis and *in vitro* expression can be used to identify critical amino acids required to maintain structure or function.³⁸ Broad tissue distribution can be determined using the cDNA clone as a probe of commercially available tissue-specific cDNA libraries. Alternatively, variant alleles can be cloned and expressed in cell culture to explore transcription, translation, or translocation processes responsible for their altered expression.

CLASSIFICATION OF THE MOLECULES THAT EXPRESS ANTIGENS

The molecules that show blood group and platelet antigens express these polymorphisms either by changes in carbohydrate or amino acid composition. Carbohydrate antigens represent residues that are attached to lipids or any number of polypeptides via N- or O-residues on amino acids. Therefore, carbohydrate antigens may be expressed on RBCs, platelets, and other blood cells. Polypeptides that express antigens are classified into 4 types. They can be either type I or type II single-pass transmembrane proteins that may or may not be glycosylated. Type I transmembrane polypeptides are oriented with their C-terminal domain within the intracellular milieu. The N-terminal domain may be external or intracellular. Type II polypeptides have the opposite orientation; their C-terminal domain to the exterior of the cell and the N-terminal within the interior. Type III, or the multi-pass type I transmembrane polypeptide, is usually indicative of transporter and channel forming molecules. Lastly, some polypeptides are linked to the plasma membrane via a GPI anchor (Figure 1).

One of the most informative ways to classify the molecules that express antigens is by their function. Functional groups are defined by molecules with similar structural features because structure often dictates function (Table 1). For example, channel forming or transporter function is based on multitransmembrane spanning proteins. The functions for some of the molecules that express RBC

antigens are not known conclusively even though the nucleotide and deduced amino acid sequences have been established. In these situations, the classification is based primarily on similar structural features expressed by other molecules whose function is known. A disadvantage of a functional classification scheme is that some molecules have more than one function. For example, band 3 is classified as a transporter yet it serves as a structural molecule because it has an attachment site for the cytoskeletal matrix through the interactions with ankyrin, protein 4.1, and protein 4.2. GPIIIa is a member of the superfamily of integrins and serves as an adhesion molecule and, along with GPIIb, serves as a receptor for fibrinogen during platelet aggregation. Other functional classification systems have included the exogenous interactions with bacteria, parasites, and other organisms. It is likely that infectious agents (eg, *Plasmodium vivax*) evolved to specifically bind human RBC molecules as a means of survival (replication) and that some blood group polymorphisms (eg, Fy(a-b-)) arose under selective pressure to circumvent infection.³⁹ Interested readers should consult other publications⁴⁰ because this topic will not be reviewed further.

Nomenclature and Antigen Systems

Most of the RBC blood group antigens have been cataloged into 29 blood group systems in accordance with the RBC membrane component on which the antigen resides. The genes have been cloned for 27 of the 29 systems (the genes for P1 and RAPH remain unidentified) with over 270 defined antigens.^{34,36,41} Antigens that do not belong to a particular blood group system are assigned to the blood group collection (201 series) or to the low-incidence (700 series) or high-incidence (901 series) antigen series as defined by the International Society for Blood Transfusion (ISBT) committee on the terminology for red cell surface antigens.

HPA systems are expressed on one of eight platelet membrane components. Seven of 8 genes that encode platelet antigens have been cloned.⁴²⁻⁴⁴ But, unlike the RBC blood group nomenclature, in which multiple antigens are assigned to the same system if they are encoded by the same gene, some platelet antigens are designated as different "systems" even though they are encoded by the same gene, and therefore, expressed on the same mem-

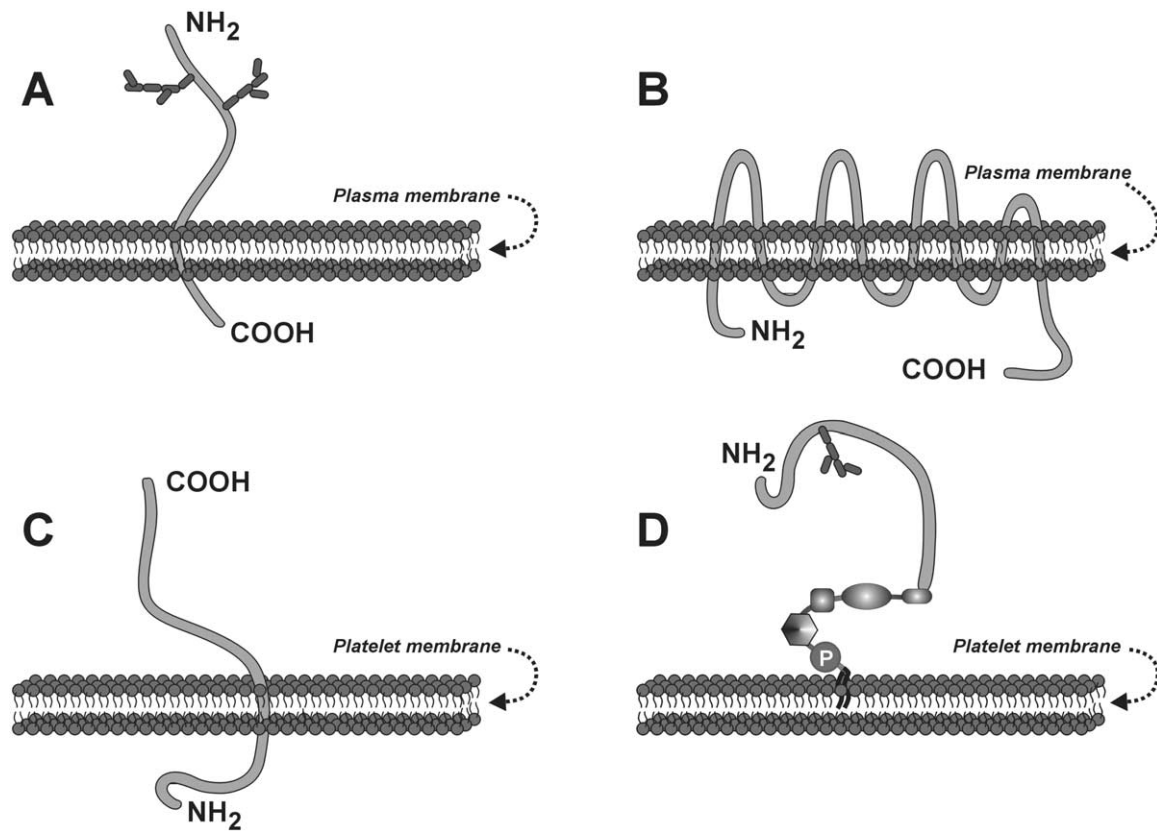


Fig 1. The classification of plasma membrane proteins. Proteins may be type I single-pass (A) or type III multiple-pass (B) transmembrane-spanning molecules (ie, their N-terminal [NH₂] end on the exofacial or endofacial side of the plasma membrane). Type II transmembrane proteins (C) are oriented with their C-terminal end on the exofacial side of the plasma membrane. Glycosylphosphatidylinositol (GPI)-anchored proteins (D) do not have a transmembrane domain and are associated with the plasma membrane via a phosphorylated (P) and acylated (O) glycerol molecule linking inositol to the C-terminal end of a protein via a glucosamine-glycan-ethanolamine bridge (□—○—□). Proteins may contain exofacial branched carbohydrate side chains (□).

brane component. Currently, there are 5 platelet antigen systems recognized by the ISBT Working Party on platelets. Two of the 5 antigen systems reside on the same platelet moiety (GPIIIa). The other 3 are expressed on GPIa, GPIb α , and GPIIb. An additional 8 antigen systems carry a workshop designation.

RBC ANTIGENS

Structural Molecules

Some of the molecules that express blood group antigens have a structural role for RBCs. Although band 3, Lutheran, Xk, and the Rh proteins all provide some structural integrity as shown by the altered shape of RBCs when these proteins are absent, they have other functions and are discussed under other headings. Glycophorins A and B do

not appear to provide structure to the RBCs; they are discussed here because of their “chaperon” function for the proper expression of band 3 on the RBC plasma membrane. It appears that GYP C and D are the only proteins that have a sole structural role for RBCs.

Glycophorin A/B. The glycophorin family consists of 2 pairs of highly glycosylated RBC proteins: GYPA/B and GPC/D. GYPA and GYPB are the pair of glycoproteins that express antigens of the MNS system. The other pair, GYPC and GYPD, is discussed in the next section. The carbohydrate residues on glycophorins have a high content of sialic acid,⁴⁵ which carry a substantial negative charge. Although not a structural function, GYPA/B likely prevent spontaneous agglutination by maintaining RBCs separate because of

Table 1. The Classification and Function of Molecules That Express RBC Antigens

Classification	Antigen System	Copy Number per RBC ($\times 10^3$)	Molecular Weight (kDa)	Function*
Structural molecules				
Glycophorin C/D	Gerbich	135/50	32,23	Cytoskeleton attachment
Glycophorin A	MNS (M/N)	1,000	36	Chaperonin
Glycophorin B	MNS (S/s)	200	20	?
Transport/channel molecules				
Band 3/AE-1†	Diego	1,000	90	Anion exchange
Rh†	RhD/RhCE	100	30–34	(NH ₄ ⁺ transport)
Aquaporin-1/CHIP	Colton	150	28–50	Water channel
Aquaporin-3	GIL	?	45	Water channel, urea transport
Kidd glycoprotein	Kidd	14	50	Urea transport
Xk glycoprotein†	Kx	1	37	(Transport)
Receptors				
Fy glycoprotein	Duffy	14	40	(Chemokine clearance)
CR1 (CD35)	Knops	1	170–280	Complement regulation
DAF (CD55)	Cromer	5–15	70	Complement regulation
ERMAP	Scianna	?	?	(Signal transduction)
Adhesion molecules				
Hermes antigen (CD44)	Indian	5–10	80	(Erythropoiesis; binds hyaluronate, collagen, fibronectin)
B-CAM/Lutheran glycoprotein	Lutheran	4	78–85	(Erythropoiesis; binds laminin)
ICAM-4/LW glycoprotein	Landsteiner-Wiener	5	42	(Cell-cell interaction, RBC turnover; binds β 1, β 2, β 3, β 5 containing integrins)
CD108	JMH	?	75–80	(Erythropoiesis; binds RGD)
Xg ^a glycoprotein (CD99)	Xg	9	29	(Apoptosis)
Neurothelin (CD147)	OK	?	54–65	(Erythropoiesis; binds LFA integrins)
Enzymes				
Kell glycoprotein†	Kell	3–6	93	(Cell signaling; cleaves Big Endothelin)
C4b/C4a	Chido/Rogers	?		Complement components
Acetylcholinesterase	Yt	10	160	(Esterase)
ADP ribosyl transferase-4	Dombrock	?	54–57	Erythropoiesis; regulate protein function
Carbohydrate Transferases				
1,3 β -GalNAc/Gal transferase	ABO	>250	—	(Prevent infection, cell development and recognition)
1,2 α -Fuc-1 transferase	Hh	variable	—	(Prevent infection, cell development and recognition)
1,3/1,4 α -Fuc-3 transferase	Lewis	?	—	(Prevent infection, cell development and recognition)
1,6 β -GluNAc-aminyl transferase	li	?	—	(Prevent infection, cell development and recognition)
1,4 α -Gal-aminyl transferase	P1	?	—	(Prevent infection, cell development and recognition)
Gobosidases	P, Pk	?	—	(Prevent infection, cell development and recognition)
Other				
MER2	RAPH	?	?	?

*The functions in parentheses indicate a proposed biological function based on amino acid sequence homology with proteins of known function.

†These molecules play a role in the maintenance of RBC structure.

their contribution to the overall net negative charge.⁴⁶ Indeed, they are the main contributor to the RBC glycocalyx, which itself may function to

protect the cells from mechanical damage because carbohydrates such as sialic acid contribute mainly to the overall negative charge of the cell.⁴⁷

Table 2. The Classification and Function of Molecules That Express Human Platelet Antigens

Classification	Antigen System	Copy Number per PLT ($\times 10^3$)	Molecular Weight (kDa)	Function*
Integrin adhesion molecules				
GPIa	HPA-5,-13w	0.8-2.8	165	Platelet adhesion; collagen receptor
GPIIb	HPA-3,-9w	50-80	116	Platelet aggregation; receptor for fibrinogen, vWF, fibronectin, thrombospondin
GPIIIa	HPA-1, -4 (HPA-6w, -7w, -8w, -10w, -11w)	50-80	90	Platelet aggregation; receptor for fibrinogen, vWF, fibronectin, thrombospondin
Leucine-rich receptor molecules				
GPIb α	HPA-2	25	143	Platelet adhesion; receptor for vWF, thrombin
GP1b β	HPA-12w	25	22	Platelet adhesion; receptor for vWF, thrombin
GPV	PI ^T (HPA-?)	12.5	82	Platelet adhesion; receptor for vWF, thrombin
Other				
GPIV (CD36)	Vis (HPA-?)	?	85	Cell adhesion; receptor for thrombospondin, collagen
CD109	Gov ^{a/b} (HPA-15w)	?	175	(Cell communication; thioester activity)

*The bracketed functions indicate a proposed biological function based on amino acid sequence homology with proteins of known function.

GYPA can be divided into 3 domains: a heavily glycosylated extracellular region (amino acids 1-72), a transmembrane region (amino acids 73-95), and a cytoplasmic region (amino acids 96-131) containing a high content of proline residues. It has one of the highest copy numbers ($\sim 10^6$ copies per RBC). Most importantly, GYPA forms a complex with band 3 and facilitates the translocation of band 3 to the RBC surface.⁴⁸⁻⁵⁰ In the absence of GYPA/B (the M^k phenotype), the intracellular trafficking of band 3 is distorted. Groves and Tanner⁵¹ suggested that the altered glycosylation of band 3, because of the absence of GYPA, does not affect the expression of band 3, but that the altered glycosylation may affect band 3 folding and the level of anion transport activity.⁵¹ Also, the interaction of GYPA and band 3 results in the expression of Wr^b. The RBCs of various GYPA/GYPB hybrid glycoproteins have been examined for expression of Wr^b. There is agreement that the GYPA interaction with band 3 lies between residues 59 to 70.^{52,53} Moreover, the loss of alanine at position 65 markedly affects Wr^b expression.⁵⁴ Lastly, GYPA may regulate complement lysis since it can inhibit the formation of C5b-7 on RBCs.⁵⁵ But complement deposition on RBCs is regulated more so by the membrane inhibitor of reactive lysis (CD59).

Glycophorin B is a minor RBC sialic acid containing glycoprotein. Glycophorin B shares amino acid identity with GYPA, the first 26 N-terminal amino acids of the extracellular domain are iden-

tical to group N GYPA, but it has a shorter cytoplasmic region. The exact role of GYPB is not known. GYPB is absent in some M^k phenotypes and in cells of the S-, s-, U- phenotype.⁵⁶⁻⁵⁸ However, the analysis of some rare low-incidence glycophorin antigens suggests that S-s- RBCs may express a small amount of a hybrid GYPB molecule.⁵⁹

Glycophorin C/D. Clearly, glycophorins C and D have a role in RBC structure. These glycophorins are isomers encoded by the same gene; glycophorin D lacks the first 21 N-terminal amino acids of glycophorin C.⁶⁰ Glycophorins C and D express the Gerbich blood group antigens and large nucleotide (exon) deletions lead to RBCs devoid of Gerbich high-incidence antigens. However, none of these deletions lead to altered function, at least not altered RBC shape. The cytoplasmic domain of GYPC/D interacts with the RBC, specifically the cytoskeleton proteins spectrin, actin, phosphoprotein 55, and protein 4.1. It is thought that GYPC stabilizes the cytoskeletal elements by acting as an adaptor protein for p55 and protein 4.1.⁶¹⁻⁶³

The Leach phenotype (the GYPC/D null phenotype) has been instrumental in the understanding of GYPC/D as structural glycoproteins.⁶⁴⁻⁶⁶ Leach RBCs lack all antigens of the Gerbich blood group system, show varying degrees of ovalocytosis to elliptocytosis and mild osmotic fragility, and have a shortened in vivo survival likely because of the mechanical constraints on RBC permeability.⁶⁷⁻⁷⁴ Individuals of this phenotype also have reduced

amounts of protein 4.1 and p55, which may be the critical proteins responsible for the reduced permeability.⁶³ Some examples of RBCs with an altered expression of GYPC/D express low-incidence antigens of the Gerbich blood group system.⁷³

Band 3/anion exchanger 1 transporter. Band 3, the most abundant RBC protein, exists as dimers that are noncovalently associated with GYPA. Both the N- and C-terminal domains of the molecule are oriented toward the cytoplasm. GYPA likely assists in the translocation of band 3 to the plasma membrane.⁷⁵ Band 3 participates in 2 and possibly 3 functions. First, the multipass membrane region of band 3 mainly transports anions but also water and glucose across the RBC membrane (see later).⁷⁶ Second, band 3 is important in the maintenance of the membrane's structural integrity because it is the primary anchor to the cytoskeleton. The N-terminal region binds to ankyrin, bridging band 3 to spectrin, and also interacts with protein 4.2. Band 3 is noncovalently associated with several intracellular glycolytic enzymes including glyceraldehyde 3-phosphate dehydrogenase, aldolase, phosphofructokinase, and catalase. A possible third function, band 3 may play a role in RBC aging and senescence. Studies on the in vivo clearance in dogs using selectively aged RBCs suggests that band 3 clustering generates an epitope leading to autoantibody binding and cell senescence by monocytes of the reticuloendothelial system.⁷⁶ In vitro studies designed to identify exogenous interactions suggest that band 3 participates in malarial invasion of RBCs because Southeast Asian Ovalocytosis (SAO) provides protection against malarial infection possibly because of a lack of membrane deformability.⁷⁷⁻⁷⁹

Rh-associated glycoprotein and the Rh complex. The Rh-associated glycoprotein (RhAG) has a role in the maintenance of RBC structure, although recent evidence suggests that it has ammonia transport activity.⁸⁰ Immunoprecipitation studies of the Rh null phenotype confirmed that at least the Rh-CcEe polypeptide and RhAG are required for expression of the "Rh complex."²⁷ The complex also requires Duffy, LW, CD47, and glycophorin B.⁸¹ Band 3 likely has a role because the Rh complex is depressed in SAO, a disorder that is associated with a lack of expression of band 3.⁸² In the regulator Rh null phenotype, the Rh complex is nonexistent either because of a defect in protein trafficking to the cell surface or in the assembly of the

complex because of the absence of RhAG. The same is true for amorph Rh null, which is because of the lack of RhD and RHCcEc proteins. The conceptual model of the Rh complex is a tetramer consisting of 2 Rh proteins and 2 RhAG proteins that are noncovalently associated with LW, CD47, glycophorin B, and Duffy.^{83,84} The morphological changes associated with the Rh null phenotype suggest that the Rh complex is linked to the cytoskeleton likely through band 3, protein 4.2, and CD47.⁸⁴⁻⁸⁶ Mild but chronic hemolytic anemia and spheromatocytosis are consistent clinical features of the Rh null phenotype, which also suggests that the Rh complex provides structure to the RBC.⁸⁷

Transporter and Channel Molecules

The molecules of this group have the distinct feature of multiple transmembrane spanning domains with N- and C-terminal ends on the cytoplasmic side of the plasma membrane (Fig 1B). This category includes the Rh proteins, band 3, aquaporins 1 and 3, Kidd, and Xk proteins. The function of this group of molecules has been shown directly or is inferred from the change in the biological characteristics of the RBC observed with the various null phenotypes.

Rh polypeptides. The Rh polypeptides are comprised of 2 highly homologous (92%) palmitylated, nonglycosylated proteins of 417 amino acids that are structurally related (~35% homology) to RhAG.⁸⁸ Hydropathy plots predict that each protein contains 12 transmembrane spanning domains. Only the RhAG has an N-glycan attached within the first extracellular loop. RHD expresses the D antigen and RHCE expresses C or c and E or e antigens, possibly from alternatively spliced transcripts.⁸⁹ The RhAG does not express blood group antigens. D differs from the other CcEe blood group antigens by as many as 39 amino acids⁴⁰; however, not all differences are found between D and the CcEe polypeptide. The D antigen is identical to C in exon 2. Yet c differs from C by 4 amino acids, 1 in exon 1 (tryptophan16cysteine) and 3 in exon 2 (leucine60isoleucine, asparagine68serine, and proline103serine). The e antigen differs from E by an alanine to proline substitution at position 226. The D antigen expresses an alanine at position 226, yet it does not express the e antigen. Some variants of the C and e antigens have amino acid substitutions that weaken their expression.

The *RHD* locus has more than 100 alleles.⁹⁰ However, the most unique feature of this blood group antigen is the complete lack of the protein in most Rh-negative individuals of Caucasian ancestry. In addition, approximately 1% of the random population expresses a weakened form of D. Based on serological and molecular studies, the weakened expression of D is divided into 2 classes. The “weak D” phenotype is characterized by the expression of single amino acid substitutions primarily in the transmembrane hydrophobic domain or within cytoplasmic regions. These molecules likely have an abnormality in protein trafficking or folding within the plasma membrane, which results in a quantitative decrease in expression. Essentially, the repertoire of epitopes displayed is normal. Individuals of this phenotype do not make anti-D on exposure to the wild-type RhD. In contrast, “partial D” variants have qualitative differences compared with the wild-type D. Individuals that express only partial D antigens have the potential to make anti-D because these molecules lack important structural epitope(s). The genetic changes for partial D’s include critical point mutations, usually in the exofacial domains of the molecule, or genetic rearrangements involving homologous regions of *RHCE*.

Various functions have been proposed for the Rh proteins.⁹¹ The most recent evidence suggests that Rh proteins function in the transport of ammonium because the Rh polypeptides and RhAG share significant sequence homology with Mep proteins, a group of multimembrane spanning ammonium cation transporter proteins in yeast.⁹² Recently, Marini and coworkers⁹³ showed that the transfection of yeast with RhAG-complemented Mep-deficient growth on selective medium containing low ammonium. RhAG complementation was specific for ammonium transport as it did not complement amino acid transport deficient nor potassium transport deficient yeast strains. The investigators also found that the expression of RhAG conferred yeast resistance to methylamine, a toxic ammonium analog. Furthermore, they showed that an Rh-related glycoprotein PDRC2, now termed RhGK and selectively expressed in human kidney, could also complement the Mep-deficient yeast. The hypothesis is that RhAG and RhGK function as ammonium transport proteins in human RBCs and kidney, respectively. The investigators propose that RhAG may promote RBC retention of

ammonium for subsequent release to the liver. They suggested that studies of ammonium levels in Rh null cells might provide support for this theory. An alternative hypothesis is that Rh proteins have lost significant transport function, but their expression is retained because they participate in the maintenance of membrane integrity.

Band 3/anion exchanger 1 transporter. Band 3 is a 93-kDa multipass transmembrane glycoprotein expressed on RBCs and on intercalating cells of the renal collecting ducts. Band 3 is encoded by anion exchanger 1, which is also known as the Solute Carrier Family 4/Anion Exchanger 1 (SLC4A1) gene, a member of the family of anion transporter genes.^{94,95} The gene encodes 911 amino acids that predict 14 transmembrane spanning domains. There is only one N-linked oligosaccharide at asparagine 642 that carries the ABH and I/i blood group determinants. Band 3 expresses the Diego blood group system antigens, which are represented by single amino acid substitutions.⁹⁶ Various other mutations that do not represent blood group antigens affect the expression of band 3 and account for one quarter of the cases of hereditary spherocytosis. In particular, SAO is associated with a 9 amino acid deletion (400-408).

Studies on the noncovalent interactions between band 3 and GYPA have shown the structural requirements for W_r^b . Initially, Bruce and coworkers^{13,48,97} showed that the low-incidence antigen W_r^a was associated with a single nucleotide polymorphism in the fourth domain of band 3, a glutamic acid to lysine substitution at position 658. Interestingly, W_r^b is dependent on the expression of both GYPA and band 3 proteins. Initial evidence suggested that W_r^b resided on GYPA because GYPA-deficient and many GYPA variant cells were $W_r(b-)$. However, earlier studies by Dahr and coworkers⁵² showed that the sequence of GYPA in $W_r(a+b-)$ individuals was unchanged from the wild type and that W_r^a did not segregate with M and N antigens. Telen and Chasis⁹⁸ showed that band 3 and GYPA coprecipitated with anti- W_r^b antibodies. Conversely, GYPA is not necessary for band 3 expression as GYPA-deficient cells (En[a-]) express normal amount of band 3 but with increased amount of glycosylation. However, the lack of GYPA affects RBC anion transport suggesting that the crucial conformational information of band 3 is lost in the absence of GYPA. Furthermore, the interaction of band 3 and GYPA

is necessary to maintain membrane deformability because the binding of anti-Wr(b) antibodies has been shown to increase membrane rigidity. It is likely because of an alteration in the noncovalent interaction of band 3 at glutamic acid position 658 with a region of GYPA spanning peptides 59 to 71.²⁷

Aquaporin-I and the Colton blood group system. Aquaporin-I (AQP-1) is a member of the major intrinsic protein family and is also known as a channel-forming integral protein (CHIP). This 28-kDa protein is expressed also on the proximal tubules and the descending loop of Henle, likely functioning to reabsorb water from the glomerular filtrate.⁹⁹ The molecule carries ABH determinants and the Colton blood group polymorphism on the first extracellular loop.¹⁰⁰ The expression of AQP-1 on RBCs is not critical for the maintenance of cell integrity. RBCs that lack AQP-1, the Co(a-b-) null phenotype, have an 80% reduction in osmotic water permeability but do not have any obvious structural pathology.¹⁰¹ It is possible that Kidd and AQP-3, or another unidentified protein transport water in the absence of AQP-1, can avoid an observable RBC or kidney functional defect. The genetic defect leading to the Co(a-b-) null phenotype has not been determined. It has been proposed that a mutated or missing nuclear transcription factor may be responsible for the defect.¹⁰²

The gene encoding AQP-1 predicts a protein with 6 membrane-spanning domains with both N- and C-terminal domains oriented within the cytoplasm of the RBC.^{103,104} Crystallographic studies have shown that RBC AQP-1 is a complex of 4 monomers. Transmembrane spanning regions 1, 2, and 6 associate more tightly as do 3, 4, and 5 allowing the more hydrophobic loops between domains 2/3 and 5/6 to overlap in an hourglass formation.¹⁰⁵ In the tetrameric complex, the hydrophobic loops are oriented inward in a ring fashion that forms the channel along with transmembrane region 2.

Aquaporin-3 and the GIL blood group system. Aquaporin-3 (AQP-3) is also a major intrinsic protein channel forming molecule and differs from the function of AQP-1 in that it can transport glycerol, water, and urea.¹⁰⁶ But, it is similar to AQP-1 in that it likely traverses the plasma membrane 6 times with both amino and carboxyl-termini located on the intracellular side of the membrane.¹⁰⁴

AQP-3 is expressed in other tissues including, kidney, liver, pancreas, lung, spleen, and prostate.¹⁰⁷ The expression of the GIL antigen on AQP-3 was identified using the RBCs from 2 individuals who had antibodies to the high-incidence antigen, GIL.¹⁰⁸ First, the 2 individuals did not express AQP-3 as determined by immunoprecipitation studies using a cross-reactive rabbit anti-rat AQP-3 polyclonal antibody. In addition, human anti-GIL failed to react by flow cytometry with COS cells transfected with their AQP-3 cDNA. Both individuals had the same G to A nucleotide substitution in intron 5 leading to the loss of exon 5 after RNA processing and a failure to express AQP-3. Detailed studies on glycerol permeability in AQP-3-deficient RBCs led the authors to propose that another protein is involved in the transport of glycerol. These observations are consistent with the fact that GIL null RBCs are not associated with any overt functional defect and that mouse RBCs, which lack an equivalent AQP-3 transport molecule, transport glycerol efficiently.¹⁰⁹ Once again, there is evidence to suggest that RBCs have evolved functional redundancy for unknown reasons.

Xk protein. The Xk protein, another potential multipass transmembrane-spanning transporter, is required for the appropriate assembly and expression of the Kell glycoprotein in RBCs.^{29,110} This is because of the disulphide bond between Xk and the Kell glycoprotein.^{28,111} There are no blood group polymorphisms associated with Xk, but anti-Kx develops after transfusion in the Xk null individual. Furthermore, acanthocytic RBC morphology and a loss of Kell, known as the McLeod phenotype, is a hallmark of the Xk null phenotype.¹¹² Xk has structural features similar to neurotransmitter transport proteins, which also have multitransmembrane-spanning domains.¹¹³ The absence of Xk, which is expressed normally in brain and muscle tissue, is likely responsible for the pathological observations including myopathy, neurogenic muscle atrophy, and a host of neurologic symptoms.¹¹⁴ The cellular functional defect responsible for these observations is unknown. For RBCs, however, it is reasonable to assume that alterations in the organization of the lipid bilayer is responsible for the changes in cell membrane structure.^{115,116} An extensive review of Xk and Kell has been published recently in this journal.¹¹⁷

Kidd glycoprotein. The Kidd (Jk) glycoprotein, the human urea transporter 11, has 10 potential membrane spanning domains with a single N-glycan attached to the third extracellular loop. The single amino acid substitution, asparagine for aspartic acid at position 280, is responsible for the Kidd antigens.¹¹⁸ The glycoprotein consists of 389 amino acids not 391 as originally reported. This is caused by a hexanucleotide deletion that is common among the random population.¹¹⁹ There are no other blood group polymorphisms with the exception of the null phenotype which produce anti-Jk3 after transfusion; Jk3 is expressed on all Kidd-positive RBCs. Jk null cells are resistant to lysis by 2 mol/L urea because these cells do not readily transport urea and thus do not become hypertonic.¹²⁰ Furthermore, the necessity for the rapid transport of urea by the Kidd glycoprotein after RBCs pass through the kidney is uncertain because the null phenotype is not associated with any hematological manifestations. Kidd is also expressed on endothelial cells of the kidney medulla. Presumably, the null defect affects expression in the kidney, but its absence does not totally impair the ability of the kidneys to excrete urea. The kidneys are able to compensate because of the expression of another urea transporter, human urea transporter 2.¹²¹ No other abnormalities are noted, apart from the fact that individuals of the Kidd null phenotype have a reduced capacity to concentrate urine.¹²²

Receptor Molecules

The binding of ligands to mature RBCs appears to have no function because cytoplasmic-signaling pathways do not exist. Some receptor molecules on RBCs function as regulators of complex systems such as complement activation or blood cytokine levels although the later function attributed to Fy glycoprotein is still speculative. It is possible that some of these receptors are functionally important in early, nucleated erythrocyte lineages or have evolved an alternate function such as participating in membrane structure (eg, LW).

The Duffy glycoprotein. The Duffy antigen is a multipass transmembrane-spanning 37- to 45-kDa glycoprotein. The deduced primary sequence comprises 338 amino acids, although the major expressed mRNA spliceoform is 336 amino acids long.¹²³⁻¹²⁵ Duffy is expressed also on endothelial cells, epithelial cells of the kidney collecting ducts, lung tissue, and Purkinje cells of the brain.¹²⁶⁻¹²⁹

The molecule has 7 transmembrane spanning domains, although it does not appear to function as a transport molecule. A major structural difference compared with other RBC channel-forming molecules is that the N-terminal region of the glycoprotein is oriented on the exofacial surface. The molecule expresses a common polymorphism, Fy^{a/b} blood group antigens, which is caused by a glycine to aspartic acid substitution at position 42 of the expressed glycoprotein. An additional amino acid change, responsible for the Fy^x phenotype that is a weakened form of Fy^b, is located at position 89 of the first intracellular loop.¹³⁰ The weak expression of Fy^x is caused by an instability of the mature glycoprotein as it translocates to the plasma membrane.¹³¹

The Duffy antigen is exploited by the malarial parasite *Plasmodium vivax* to infect RBCs. Duffy is required to the extent that the Fy(a-b-) phenotype predominates in West Africa because it confers resistance to malarial invasion. The West African Fy(a-b-) phenotype is caused by a mutation in the GATA-1 box of the promoter region of the FYB allele.^{124,132} The lack of expression is specific for RBCs; expression is not affected in other tissues. Therefore, the Fy(a-b-) individual is tolerant to Fy^b. More recently, the Fy(a-b-) phenotype has been found in the Southeast Asia region of Papua New Guinea. The mutation is an identical T to C transition in the GATA-1 promoter site at position -33 but is linked to the FYA allele rather than FYB. The authors suggested that, as with the RBC silent FYB allele, the FYA null arose by the selective pressure of *P. vivax*.¹³³ A homologous mutation leading to the Fy(a-b-) phenotype has not been documented in Caucasians. The few examples are characterized by a 14-nucleotide deletion in the coding region or by 1 of 3 nonsense point mutations that introduce a stop codon.^{132,134} These individuals represent true null phenotypes and make anti-Fy3 when transfused. The Fy³ epitope maps to the third extracellular loop and lies in close proximity to the Fy^{a/b} and the chemokine-binding region.¹⁸

The function of Duffy is controversial. A comparison of the genomic sequence shows that it has sequence homology to the family of interleukin (IL)-8 receptors known as chemokines.¹³⁵ Chemokines are generally expressed on nucleated cells and are coupled to G-protein cell-activation pathways. Duffy is unique in that it will bind to C-C

and C-X-C classes of chemokines.¹³⁶ The lack of an activation pathway in mature RBCs and the promiscuous binding led investigators to suggest that Duffy expression on RBCs act as a biological “sink” to clear these inflammatory cytokines from the circulating blood.^{137,138} On the other hand, the existence of the Fy(a-b-) RBC phenotype suggests that it is unnecessary for normal RBC development and function. Individuals of the true Duffy-null phenotype have no obvious hematological or immunological abnormalities. In support of these observations, the FY knockout mouse shows no immune system or tissue abnormalities when compared with wild-type mice except for a blunted neutrophil response on exposure to bacterial lipopolysaccharide.¹³⁹ However, the susceptibility of FY knockout mice to *Staphylococcus aureus* was identical to wild-type mice. These observations led the investigators to conclude that Duffy is functionally redundant. Similar studies by Dawson and coworkers¹⁴⁰ revealed an increase in inflammatory infiltrates in the lung and liver of FY knockout mice challenged with lipopolysaccharide. In similar studies of patients with Sickle cell disease, elevated levels of IL-8 were noted in acute chest syndrome when compared with Sickle cell patients treated for vascular distress.¹⁴¹ Of the 20 patients examined, 14 had elevated levels of IL-8 and 19 of them were Fy(a-b-). Whether the frequency of Fy(a-b-) in this small group of patients reflects a risk for the development of acute lung syndrome is controversial as the frequency of Fy(a-b-) among West African blacks is approximately 75%. Studies of membrane cofactor protein (MCP)-1 levels in healthy adults show higher levels in men versus women and higher levels in individuals who are Fy antigen positive versus Fy(a-b-).¹⁴² Whether the variation in MCP-1 levels poses a risk for certain chemokine-associated diseases is unknown.

Complement receptor 1: Knops blood group system. The complement receptor 1 (CR1) glycoprotein consists of up to 30 repeated and disulphide-bonded domains called complement control protein (CCP) modules. These modules, also termed short consensus repeats (SCRs), are organized into 4 regions called long homologous repeats each region consisting of 7 SCRs.¹⁴³ Most importantly, CR1 copy number on RBCs varies greatly among healthy individuals. Less than 100 copies per RBC are not detected using conven-

tional blood group antibodies for the high-incidence antigens expressed on this receptor molecule including Knops (Kn), McCoy (McC), Swain-Langley (Sl), and York (Yk).^{144,145} Variation in copy number is the likely reason for the inconsistent reactions seen for antibodies of this blood group system.¹⁴⁶ Recently, Moulds and coworkers¹⁴⁷ mapped the McC and Sl blood group epitopes to the LHR D region. They found that McC was associated with a lysine to glutamic acid substitution at position 1590 and that the Sl polymorphism was caused by an arginine to glycine substitution at position 1601. Concordance between the genotype and phenotype was not 100% in the populations tested, but these observations were attributed to the poor expression of CR1 in almost all instances.¹⁴⁷

CR1 functions as a receptor for C3-convertase complexes. Cell-bound C3b is rapidly cleaved to C3dg, and it appears that CR1-bound C3 can be cleared without RBC destruction.¹⁴⁸ Monocytes and neutrophils also express CR1 and are able to phagocytose complement coated cells. Furthermore, RBCs have been shown to have a buffering effect on neutrophil degranulation and monocyte phagocytosis because of the binding of C3 coated immune complexes.^{149,150}

Decay accelerating factor: Cromer blood group system. Decay accelerating factor (DAF) is a glycosylated protein that regulates complement activation and whose structure is based on the complement control protein domains found for CR1. However, DAF has only 4 SCRs and is attached to the cell membrane via a GPI-link. No putative transmembrane hydrophobic region is identified by hydropathy analysis. The molecular bases for all but one of the 10 Cromer antigens have been determined.¹⁵¹⁻¹⁵³ Single nucleotide polymorphisms have been localized to the first, third, and fourth SCRs. No functional differences have been reported for the various blood group polymorphisms. The Cromer null phenotype is caused by a nonsense mutation resulting in a premature stop in translation.¹⁵⁴

DAF is also found in plasma and other body fluids and prevents the activation of C4b2a by inhibiting the deposition of C3b on the surface of RBCs.¹⁵⁵ Together with MCP, DAF catalyzes Factor I-mediated cleavage of C3b that binds to RBCs.¹⁵⁶ The distal SCRs are responsible for regulating complement activation.¹⁵⁷

Adhesion Molecules

Adhesion molecules have no obvious function on mature RBC but may participate in cell-to-cell or cell-matrix interaction during erythropoiesis or possibly during hematopoietic cell maturation. None of the null phenotypes have conclusively shown that these adhesion molecules are an absolute requirement for RBC or other hematopoietic cell maturation. Some of the adhesion molecules on RBCs (ie, Lutheran, LW, Ok [CD147], and Bg) are members of the immunoglobulin gene superfamily of glycoproteins with homology based on a disulphide-bonded immunoglobulin-like domain consisting of approximately 100 amino acids.

CD44: Indian blood group system. CD44, the RBC receptor for hyaluronic acid, is expressed on many tissues and in various isoforms as a result of alternate splicing of the primary RNA transcript. The RBC isoform is a single transmembrane, 320 amino acid glycoprotein. The Indian (In)^{a/b} blood group antigens are defined by a proline to arginine substitution at position 46 of the mature glycoprotein. The substitution is within one of the 3 binding motifs for hyaluronic acid.¹⁵⁸ Site-directed mutagenesis studies and the rare blood group phenotype In(a+b-) have shown that the motif encompassing the In^a polymorphism does not bind hyaluronic acid. However, the 2 other hyaluronan-binding motifs are not affected and seem to function normally.¹⁵⁹

CD44 may function in adherence of precursor RBC and lymphoid lineages to the marrow stroma.^{160,161} The CD44 null phenotype has been reported in a single case of congenital anemia associated with dyserythropoiesis.¹⁶² Whether CD44 is responsible for the dyserythropoiesis is uncertain because the RBCs were also Colton null.¹⁶³ The characteristics of the null phenotypes indicate that the defect likely involves an erythrocyte-specific nuclear transcription factor because CD44 was expressed at normal levels in other tissues.

Basal cell adhesion molecule: Lutheran blood group system. The Lutheran glycoprotein is expressed on RBCs as 2 isoforms. Each has 5 immunoglobulin-like domains and differs in the C-terminal region; the cytoplasmic domain of the 85-kDa isoform has an additional 40 amino acids. The 78-kDa smaller isoform is identical to basal cell adhesion molecule.^{164,165} The Lutheran glycoprotein

has a number of structural features including a potential RGD integrin-binding site, and laminin-binding motif on the extracellular portion of the molecule, and a Src-binding domain, di-leucine motif, and potential phosphorylation site on the 40 amino acid cytoplasmic region of the 85-kDa isoform. The cytoplasmic region of Lutheran also interacts with the membrane cytoskeleton.¹⁶⁶ The Lutheran blood group antigens have been mapped with respect to their immunoglobulin-like domains.¹⁶⁷ There has been no systematic effort to determine whether any of the Lutheran blood group polymorphisms affect laminin binding, especially in those regions considered essential for binding.

Although other molecules on RBCs can bind laminin, Lutheran appears to be the natural receptor because laminin fails to bind in the absence of Lutheran.¹⁶⁸ There is disagreement on the region responsible for laminin binding. In 3 independent studies, the first 3 N-terminal and the fifth immunoglobulin-like domains are necessary for laminin binding.^{166,168,169} This work has achieved much interest because the Lutheran antigen expression and RBC binding to laminin is increased on Sick cell. This observation lead Telen and coworkers¹⁷⁰ to speculate that laminin is responsible for the binding of RBCs to vessel wall subendothelium during vascular occlusion in Sick cell crisis.

Intercellular Adhesion Molecule (ICAM)-4: LW blood group system. The LW antigen was named after Landsteiner and Wiener for their work on this Rh complex-associated antigen.¹⁷¹ The structure of the LW glycoprotein is based on immunoglobulin-like domains of ~100 amino acids that are each stabilized by a single disulfide bond. The structure shares identity with intercellular adhesion molecules, and ICAM-2 is the closest member. The fact that LW is dithiotreitol sensitive is consistent with a molecule that carries disulfide bonds.¹⁷² LW is expressed on RBCs but is also found on other blood cells including T and B cells.¹⁷³ Thus, an adhesion role for LW is conceivable, but whether it functions as an adhesion receptor on RBCs is still speculative. LW has been shown to bind to the very late activation (VLA) antigen $\alpha 4/\beta 1$ and the $\alpha v/\beta 5$ vitronectin receptor. These findings suggest that LW may participate in RBC maturation because of this adhesive property.^{174,175} The binding of LW to a similar integrin, $\alpha IIb/\beta 3$, was examined by Hermand and coworkers.¹⁷⁶ They showed that

platelets adhered better to ICAM-4-positive than ICAM-4-negative (LW null cells) immobilized RBCs and platelets deficient in GPIIb/IIIa did not bind to an ICAM-4-coated microtitre wells. In addition, CHO cells were transfected to express GPIIb/IIIa and were able to adhere to microtitre wells coated with an ICAM-4-Fc fusion protein.¹⁷⁶ There is no in vivo evidence that platelets and RBCs interact in vivo, but these molecules may participate in pathological status such as vaso-occlusion in Sickle cell disease.

The molecular basis for the LW^a/LW^b polymorphism is a single-nucleotide mutation (A>G at position 308) that does not appear to have any functional significance.¹⁷⁷ The LW protein is part of the Rh complex and is expressed on D-negative RBCs at about 1/2 the copy number per cell. In a longstanding scientific debate, the LW antigen was thought to be the factor responsible for classical hemolytic disease of the newborn because rabbit antibodies to the protein did not react with maternal RBCs. However, LW is not the "Rh factor" but is intimately associated with the Rh antigens because it is not expressed in the Rh null phenotype.¹⁷⁸ The lower antigen copy number and the inability of the rabbit antibody to agglutinate D-negative RBCs are likely reasons for the confusion surrounding the role of LW in HDN. The converse is not true, however, because LW null cells, characterized by the LW(a-b-) phenotype, do not lack Rh antigens.¹⁷⁹

CD147 (neurothelin, basigin): OK blood group system. The OK blood group system represents another immunoglobulin gene superfamily molecule. Similar to LW, the extracellular domain is predicted to form 2 immunoglobulin domain-like structures that are disulfide bonded.¹⁸⁰ The OK glycoprotein is identical in structure to the human basigin molecule (CD147), a leukocyte activation antigen. The OK(a-) phenotype found only in Japanese is the result of a single amino acid substitution.¹⁸¹ The function of the molecular is uncertain. But OK is widely expressed on blood cells, epidermis, and at the blood brain barrier.^{87,182,183} Studies with basigin knockout mice showed that it is required for embryo implantation and that it is expressed during spermatogenesis.¹⁸³ Reverse-transcription polymerase chain reaction analyses in man confirmed that basigin is expressed in testes during spermatogenesis and is also expressed in azoospermic men with the Sertoli cell only syn-

drome.¹⁸⁴ Basigin may bind mannoside containing glycoconjugates.¹⁸⁵

Bg antigens. Although not true blood group antigens, the Bg antigens are another immunoglobulin gene superfamily class of molecules that are acquired from the plasma and are soluble forms of HLA class antigens. The Bg antigens are divided into separate subgroups on the basis of predefined reaction patterns. Although anti-Bg antibodies are directed to HLA-like antigens, they are not true anti-HLA antibodies and they do not cause transplant rejection.

CD99: Xg blood group system. The Xg blood group is the second of 2 sex chromosomal blood group systems and is expressed in both men and women. Xg(a+) is the only antigen and has a high frequency of expression among humans. The Xg(a+) versus Xg(a-) blood group is defined by a difference in the level of the Xg antigen on the surface of the erythrocyte rather than a different gene product.^{186,187} Xg antigen escapes inactivation because of the expression of the gene, MIC2, which lies telomeric and in the pseudoautosomal region of the X chromosome.¹⁸⁸ Also known as CD99, MIC2 is expressed on all cells. A homologous gene also resides in the euchromatin region of the Y chromosome and is expressed independent of the X chromosome MIC2.¹⁸⁹ CD99 is also known as E2, which is an adhesion molecule expressed on T cells. Recently, a portion of CD99 was shown to activate a caspase independent apoptosis pathway in T cells.¹⁹⁰

CDw108: JMH blood group. JMH is a GPI-linked blood group antigen whose antibodies are typically of high titer but low avidity. The antigen is expressed on RBCs, some activated lymphocytes, neurons, epithelia, and testes.¹⁹¹⁻¹⁹³ JMH molecules are part of plasma membrane complexes that are associated with intracellular protein kinases.¹⁹¹ Like other GPI-linked molecules, JMH on RBCs and other cells may be as a receptor that plays a role in signal transduction.

Erythrocyte membrane-associated protein: Scianna/Radin blood group system. Human Erythrocyte membrane-associated protein (ERMAP) is expressed exclusively on erythroid cells. It is predicted to have only one extracellular transmembrane Ig-like domain. The intracellular region has a conserved B30.2 domain and multiple kinase-dependent phosphorylation consensus motifs.^{193,194} Therefore, ERMAP is likely a receptor/signal

transduction molecule specific for erythroid cells.¹⁹⁵ Both the Scianna and Radin blood groups were mapped to ERMAP.¹⁹⁶

Enzymes

Metalloproteinase: Kell blood group system. The Kell glycoprotein is a type II single-pass transmembrane glycoprotein and has a short intracellular N-terminal region and a large cysteine-rich C-terminal extracellular domain.¹⁹⁷ Kell shares identity with the M13 or neprilysin, subfamily of zinc endopeptidases and has the enzymatic activity necessary to cleave big endothelin 3.^{117,198} The endopeptidase conserved region of Kell is encoded by exon 16, a small 68 basepair exon of *KEL*. Kell lacks a residue equivalent to Arg102 of neutral endopeptidase and, therefore, is unlikely to have dipeptidylcarboxy peptidase activity but rather functions as an endopeptidase. The Kell glycoprotein may participate in the early stages of hematopoiesis or cell lineage determination.¹¹⁷

The expression of Kell on RBCs is unique among the M13 endopeptidases in that it is covalently linked to the Xk glycoprotein via a disulfide bond Cys72 near the transmembrane region. In fact, expression of Kell is dependent on the Xk protein and, together, the 2 molecules are necessary for Kell blood group system.¹⁹⁹ The Kell-null phenotype is characterized by an absence of Kell glycoprotein and an enhanced expression of Xk protein on the surface of RBCs. Typically, the Kell gene is silenced and the RBCs of this phenotype have normal structure. The McLeod phenotype is characterized by the loss of expression of Xk. Many individuals of the McLeod phenotype have a gross deletion of the X chromosome such that other genes in close proximity are also deleted. Some deletions include genes associated with chronic granulomatous disease, Duchene's muscular dystrophy, and retinitis pigmentosa. The absence of expression of Xk results in acanthocytosis and is such a typical feature of the McLeod phenotype that acanthocytes seen on blood films usually results in a screen for presence of these other disorders.²⁰⁰

Acetylcholine esterase: Cartwright blood group system. The Cartwright blood group system is carried on the acetylcholine esterase molecule.²⁰¹⁻²⁰³ The molecule isolated from RBCs has esterase activity, but its function in RBCs is uncertain. The Yt(a-b-) RBC has no obvious abnormalities.

Acetylcholine esterase is another example of a RBC GPI-linked protein; however, it is expressed as a conventional type I transmembrane protein on neuronal cells, likely from alternate splicing of the mRNA.²⁰¹ It exists as a dimer on RBCs, and early studies showed that various isoforms can be extracted from RBC membranes.^{204,205}

Adenosine diphosphate ribosyltransferase: Dombrock blood group system. The Dombrock blood group antigens also reside on a GPI-linked glycoprotein.²⁰⁶ Additional antigens carried on the molecule are Gregory, Holley, and Joseph.^{207,208} A location of a candidate gene, which was defined on the basis of longstanding karyotypic and biochemical features of the molecule,^{206,209-211} was identified using an erythroid expressing sequence tag.²¹² The molecule possesses an adenosine diphosphate-ribosyltransferase motif, an enzyme that acts as a regulator of protein function through post-translational modification of the addition of the adenosine diphosphate-ribose moiety to a target molecule. But enzymatic activity for the RBC molecule was not detected. The polymorphism leading to the Do(a/b) antigens was identified at the time the candidate gene was sequenced. The molecular mechanisms associated with the Hy(-) and Jo(a-) phenotypes help explain some of the observations for the varied expression of these antigens. The loss of Jo(a) in Hy-negative RBCs and the weakened expression of Hy in the Jo(a-) phenotype may be due to the close proximity of the amino acid substitutions for these 2 antigens. Moreover, the single amino acid substitution, leucine300valine, is likely responsible for the weak expression of both Do(b) and Gy(a).²⁰⁷ Various mutation events lead to the Do null phenotype with no obvious deleterious effect to RBC structure or function.²¹³⁻²¹⁵

Carbohydrate Transferases

Glycosyltransferases, ABO, H, Se, Le, and I blood group systems. The glycosyltransferases receive the most notoriety and respect among the blood group systems. Their discovery laid the foundation of transfusion immunology, and the rules of compatibility were critical to the safe transfusion of blood. The A, B, and H blood group substances are carried on glycolipids, glycoproteins, and also exist as free oligosaccharides. Depending on the presence of a secretor gene (Se), blood group substances are also found in secretions of which saliva is the most often cited because of

accessibility. Most of the ABH antigens on RBCs exist as type 2 chains attached to proteins with a majority of expression associated with band 3.²¹⁶⁻²¹⁸ The ABH antigens are characterized by single sugar residues, termed the immunodominant sugar, which are linked to a carbohydrate chain. One single gene is responsible for the addition of fucose to express H substance, which is the precursor to A and B antigens. A and B antigens are controlled by a single locus of codominantly expressed glycosyltransferase alleles. Group O is commonly the result of an amorphous allele at the ABO locus. These transferases can be found in RBC membranes, free in serum or plasma, and in a number of body fluids. ABH antigens and other carbohydrates may contribute to the overall net negative charge (the glycocalyx) of RBCs so that they repel one another. In addition, they may provide some spatial organization to glycoproteins and glycolipids by virtue of their charge.^{219,220}

The function of ABH and other carbohydrate antigens is unknown. ABH antigens show distinct temporal expression during ontogeny and, along with other carbohydrate antigens like Lewis, can either be repressed in some malignancies or overexpressed in others.²²¹ How the major ABO blood groups came to be is still a mystery. But one fact stands out. AB subjects are most resistant to cholera and secretor status may provide additional protection.²²²⁻²²⁴ Thus, it is conceivable that both A and B blood group alleles evolved and were maintained through evolution by the selective advantage of resistance to disease. It is also known that Group O individuals are more resistant to malaria.²²⁴ Therefore, it is possible that a quasiselective advantage exists for various carbohydrate antigens in addition to their expression during embryogenesis and tissue development.

The gene responsible for I/i blood group expression has been cloned and sequenced. The 2 antigens are structurally different. The i antigen is a linear poly-N-acetyllactosamine, whereas the I blood group antigen is branched. Fetal "i" is converted to I by β 1,6-N-acetylglucosaminyltransferase (IGnT1 or IGnT isoform A). It appears that IGnT3 (IGnT isoform C) is active in bone marrow and responsible for the expression of I on RBCs.^{226,227}

Paraglobosidases and globosidases: P1 and P/Pk blood group systems. For many years, the biochemistry of the P1, P, and Pk phenotypes were

not well understood. The antigens did not appear to be expressed on a single substrate, yet anti-P+P1+Pk mixture of antibodies were present in the p phenotype.²²⁸ The antigens were grouped together for these reasons. However, it was realized that P, Pk were expressed on a globoside glycolipid and P1 was expressed on a different glycolipid.²²⁹ Thus, P1 and P antigens arise from the enzymatic action of different transferases. P1 arises from the action of 4- α -galactosyltransferase on a type 2 paragloboside substrate, the same substrate on which ABH antigens are structured. The P antigen arises from the action of the globoside enzyme, 3- β -N-acetylgalactosaminyltransferase, which acts on the Pk antigen, a trihexose ceramide-based molecule.²³⁰ Of note, Pk also is the result of the action of a 4- α -galactosyltransferase. This enzyme may be different from the one responsible for the synthesis of P1 because it acts on lactosylceramide to produce the Pk antigen.²³¹ However, both P1 and p map near each other on chromosome 22q11 and 22q13.^{232,233} Also, p individuals that do not express P, Pk, and P1 show insertion and deletion mutations in 4- α -galactosyltransferase.²³⁴

Other (Orphan Molecules)

C3/C4: Chido/Rogers blood group systems. Chido and Rogers blood group antigens are fragments of complement component C4A and C4B, respectively, but are not true RBC-derived antigens.²³⁵⁻²³⁷ They are absorbed onto the RBC independent of classical complement activation.²³⁶ It is uncertain whether there is a biological reason for their adsorption onto RBCs. They do not have any clinical significance in transfusion compatibility other than the fact that they represent nuisance antibodies in antibody investigations and must be neutralized to exclude other clinically significant antibodies.^{174,238,239}

MER2: RAPH blood group system. MER2 was first identified as a blood group antigen using 2 murine IgG monoclonal antibodies. Over 92% of RBCs tested were MER2+. The antigen is also expressed on fibroblasts, and some carcinoma cells as RBCs were not used to produce the antibodies in mice. Subsequently, human antibodies to the MER2 were identified, but they are rarely encountered. It is not known whether the moiety that expresses MER2 is absent from MER2- RBCs. But, because of the very low frequency of anti-MER2 antibodies among humans, it is conceivable

that MER2 is absent from RBCs and expressed on other tissues. Thus, only the rare MER2 null would be capable of making antibodies.

PLATELET ANTIGENS

HPA systems comprise the list of polymorphic plasma membrane molecules that are immunogenic in humans. Alloantibodies appear as a result of transfusion or pregnancy, and the platelet antigen systems are numbered in order of their identification. The genes that encode the antigens are named in accordance with the International System for Human Gene Nomenclature.^{240,241} However, the ISBT numbering does not conform to the “one protein one system” rule that applies to RBC terminology. Many of the platelet antigen “systems” are single amino acid variants of the same molecule that express another platelet antigen system.

Human platelet antigens are expressed on 1 of 8 membrane components (Table 2). There are 5 platelet antigen systems recognized by the ISBT Working Party on Platelets and at least 8 other antigen systems that carry a workshop designation. Another 3 systems will likely receive workshop designation in the near future. The genomic sequence or exon/intron boundaries for 7 of the 8 genes have been sequenced. Only the Gov system (HPA-15w) remains to be sequenced and is likely represented in the GenBank ordered contiguous sequence NT_007299.^{43,242,243} All antigen systems are the expressed products of single nucleotide polymorphisms with the exception of Oe^a, which is an in-frame trinucleotide codon deletion. Unlike RBC alloantibodies, the antibodies that arise from platelet transfusions for inherited platelet hemostatic defects including null phenotypes such as Glanzmann’s thrombasthenia are not given antigen designations. The ISBT recognized HPA systems are expressed on glycoprotein (GP) IIIa (HPA-1 and HPA-4), GPIb α (HPA-2), GPIIb (HPA-3), and GPIa (HPA-5). CD109 (Gov^{a/b}) is the only other system for which alloantibodies have been characterized to reciprocal antigens.^{244,245} Genomic DNA-based Gov^{a/b} genotyping is possible since the exon and the flanking intron regions containing the Gov-associated SNP was sequenced.²⁴⁶

There are 4 null phenotypes for the molecules that express platelet antigens. GPIV is expressed on platelets, monocytes, and endothelial cells. Anti-Nak^a antibodies are produced in some GPIV-null individuals, but there is no apparent functional

abnormality for platelets lacking this molecule. Three other null phenotypes are associated with mild to severe bleeding disorders.^{240,241,247,248} A deficiency of the GPIb $\alpha\beta$ /V/IX complex is seen in the giant platelet syndrome, also known as Bernard Soulier syndrome, and is the only defect associated with an obvious physiological change observable using light microscopy. Glanzmann’s thrombasthenia is characterized by a deficiency or altered expression of GPIIb/IIIa. Lastly, the loss of GPIa/IIa results in a mild bleeding disorder in which platelets fail to bind collagen. There are many other inherited defects that affect platelet function.²⁴⁷ But none of the defects are localized to molecules that defined HPAs.

The biochemical analyses of the molecules that carry platelet antigens have been investigated in light of platelet adhesion, activation, and aggregation. Therefore, much is known about the function of these molecules. CD109, which carries the Gov antigen system, is the only exception and its role on platelets is not known. Most of the molecules that express platelet antigens are members of the integrin family of receptor molecules and are obligate components for normal platelet adhesion and aggregation. Integrins are a family of heterodimeric cell surface glycoproteins that have adhesive properties to other molecules expressed on cells, to intracellular matrix proteins, and to some soluble ligands. Generally, an integrin is referred to as an “adhesion molecule” in the context of its binding to matrix proteins or other molecules on another cell and as a “receptor” relative to a soluble ligand that it binds. The integrins are grouped into subfamilies on the basis of a common β -chain. For example, platelet GPIa/IIa, or $\alpha 2\beta 1$ integrin, is 1 of 6 members of the $\beta 1$ -containing VLA proteins expressed on lymphocytes, platelets, and fibroblasts. The remaining 5 members differ in the expressed α chain.

During the process of blood clotting, platelets first adhere to subendothelium exposed as a result of vessel wall injury. Initially, the integrin-containing complex comprised of GPIb $\alpha\beta$ /GPV/GPIX binds von Willebrand factor exposed within the subendothelium. Additional adherence is the result of the interaction of GPIa/IIa with exposed collagen. Adhesion is followed by platelet intraplatelet signaling, platelet shape change, and aggregation. Intracellular signal transduction before aggregation causes a conformational change in GPIIb/IIIa so

that it can bind fibrinogen with high affinity. Once activated, platelets release the contents of their granules. Other platelets near the microenvironment of the site of injury, adhere, become activated, and are recruited into the growing platelet aggregate. Of all the molecules that play an important role in platelet adhesion and aggregation, HPA-1b- on GPIIIa and HPA-13bw (Sit^a) on GPIa show functional variation.

Integrin Adhesion Molecules

Glycoprotein Ia (integrin $\alpha 2$): HPA-5 system. Glycoprotein Ia is a VLA protein first identified on lymphocytes. It is noncovalently associated with GPIIa, a $\beta 1$ integrin. GPIa was first cloned and sequenced from lung fibroblasts. The sequence analysis showed that it has identity with the other VLA molecules primarily because of a 191 amino acid "I domain," which is thought to be the region that interacts with collagen.^{247,249} The molecule has a short cytoplasmic domain, and this heterodimeric integrin functions as a signaling molecule on platelets. Two silent single nucleotide polymorphisms have been found within the coding region of GPIa. The C807T polymorphism is associated with variable GPIa/IIa copy number. The 807T variant has higher platelet expression than the 807C variant. More importantly, the C807T SNP is associated with nonfatal myocardial infarction and stroke in young adults.^{250,251}

Santoso et al²⁵² identified an antiplatelet alloantibody from an individual and characterized the molecular basis for the expressed antigen, HPA-13bw. Collagen aggregation studies using HPA-13(bw+) platelets showed a reduced response indicating that methionine rather than threonine at position 799 of GPIa reduces the interaction of collagen within the I domain region (Fig 2). It is possible that reduced collagen binding results in less in vivo platelet activation and the reason for the presence of this allele. However, the allele frequency ($p = .25$) suggests either a recent single-nucleotide polymorphism or a relatively low selective advantage associated with its expression.

Glycoprotein IIIa (integrin $\beta 3$): HPA-1 and HPA-4 systems. GPIIIa exists as a non-covalent complex with GPIIb and this molecule belongs to a class of integrins that bind cell several adhesion molecules, matrix proteins, and ligands including fibrinogen, von Willebrand factor, fibronectin, and thrombospondin. GPIIIa is also the β subunit of the

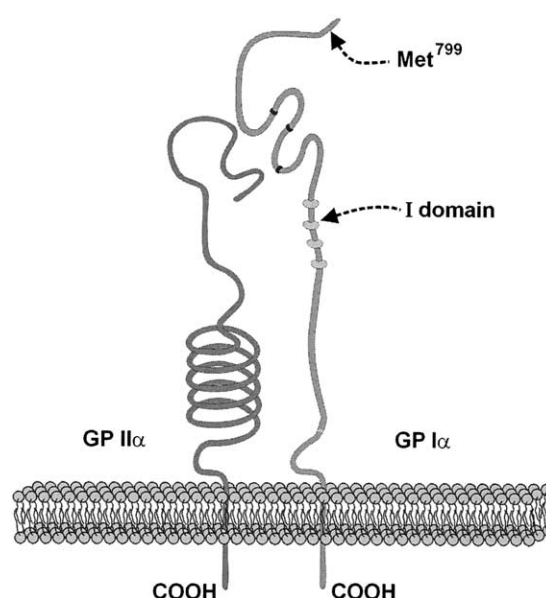


Fig 2. Schematic diagram of the platelet GPIa/IIa complex showing its orientation in the plasma membrane, the I domain (lower arrow), and the position of the methionine 799 substitution (upper arrow). Although the HPA-13bw maps to amino acid 799, the Sit^a conformational epitope likely includes the I domain region because the phenotype is associated with decreased collagen binding.

vitronectin receptor ($\alpha v/\beta 3$) and the orientation in the plasma membrane is likely altered because of the different associated alpha subunit. GPIIIa has a 26 amino acid signal peptide, a large extracellular glycosylated region containing 4 cysteine-rich tandem repeat domains of 33-38 residues and a short intracellular domain.²⁵³ GPIIIa is also expressed on endothelial cells noncovalently with the GPIIb or the αv integrin. The human platelet antigens are expressed on endothelial cells, but the clinical significance of these alloantibodies in plasma infusions or graft rejection in alloimmunized individuals is unknown.²⁵⁴

The most common form of platelet alloimmunization is to HPA-1a (also known as Zw^a or PI^{A1} antigen). A leucine to proline substitution at position 33 defines the HPA-1a/b system and is the product of a T196C single nucleotide polymorphism in exon 3 of GP3A. However, the epitope itself is more difficult to define. GPIIIa has 28 internal disulfide bonds in the exofacial domain. Recombinant technology was used to show that a minimum of 66 amino acids are required for the expression of the HPA-1a/b epitope.²⁵⁵ However,

the cysteine26-cysteine38 disulfide bond and the distal amino acids 288-490 are required for the optimum orientation of the epitope.²⁵⁶

In 1996, Weiss and coworkers²⁵⁷ identified a number of inheritable risk factors for myocardial infarction. Surprisingly, the HPA-1b allele was associated with an increased risk of myocardial infarction. More than 40 studies have examined the role of HPA-1b for risk for cardiovascular disease because the identification of HPA-1b as a potential thrombotic risk factor. A recent meta-analysis concluded that although HPA-1b is associated with an increased risk for coronary artery disease, the odds ratio is very low (1.31, 95% confidence interval 1.10-1.56).²⁵⁸ The HPA-4 polymorphism is within the RGD-binding domain of GPIIIa, but the single amino acid substitution does not appear to have an effect on ligand specificity or binding.²⁵⁹

Glycoprotein IIb (integrin $\alpha 2b$): HPA-3 system. GPIIb consists of an α - and β -chain disulfide-linked molecule that is noncovalently associated with GPIIIa. The α and β subunits of GPIIb are derived from a single precursor molecule.²⁶⁰ Post-translational enzymatic digestion results in the formation of the 2 disulfide-bonded chains. In Glanzmann's thrombasthenia, both GPIIb and GPIIIa are either aberrantly expressed or deficient. The mechanism responsible for the absence of both molecules is unknown because the disorder is characterized by a gene defect in either gene. A defect in either molecule may affect the interaction of a regulatory element required for expression or may affect trafficking or the stability of the other molecule. Glanzmann's thrombasthenia caused by a defect in GPIIb does not affect the expression of the vitronectin receptor. Therefore, the presence of the vitronectin receptor in the absence of GPIIb/GPIIIa indicates a defect in GPIIb.

The HPA-3 platelet antigen system resides on GPIIb, and the phenotypes have a high variability in frequency among different ethnic populations.²⁶¹ HPA-3 and HPA-1 show linkage disequilibrium because both genes reside in the region of chromosome 17q21.32.²⁶² There have been a few studies on the risk of coronary artery disease related to the HPA-3 polymorphism, which have yielded conflicting results.^{263,264}

Leucine-Rich Family of Receptor Molecules

Glycoprotein Iba: HPA-2 system. GPIb α is associated with GPIb β , GPV, and GPIX to form the

platelet receptor adhesion complex in a 2:2:1:2 ratio. This complex mediates the initial events of platelet adhesion (ie, the binding of resting platelets to von Willebrand Factor). The complex also binds thrombin released from platelets or generated via the classical coagulation cascade. GPIb α and GPIb β are covalently linked by a disulfide bond. These glycoproteins are members of the leucine-rich family of glycoproteins.²⁶⁵ GPIb α contains 7 leucine-rich tandem repeats, 2 globular hydrophilic regions, a classical transmembrane domain, and cytoplasmic tail. The HPA-2 system is expressed within first the N-terminal hydrophilic region.^{266,267} GPIb α is the primary molecule that binds vWF. It also shows a 13-amino acid size polymorphism near the transmembrane domain that is in linkage disequilibrium with HPA-2. The size variation is caused by a tandem nucleotide repeat that results in the expression of 3 isoforms that differ by ~ 6 kDa mainly because of the 5 potential O-glycosylation sites that occur in each repeat.^{268,269} It is thought that size differences change the distance that GPIb α extends from the platelet surface as much as 30 angstroms. However, the size polymorphisms do not affect in vitro thrombus formation²⁷⁰ but have been linked to coronary heart disease.²⁷¹

GPIb α also has a single nucleotide polymorphism in the Kozak sequence (position -5) that regulates translation of the mRNA. This polymorphism renders one sequence more homologous to the consensus Kozak sequence and results in an increase in levels of GPIb α expression. It is thought that increased expression may relate to increased platelet responsiveness to ligands.^{272,273}

Glycoprotein Ib β . GPIb β is a 22-kDa small-molecular-weight glycoprotein that is disulfide linked to GPIb α . It is a member of the leucine-rich family of glycoproteins, but it contains only one leucine motif. The N-terminal region of GPIb β is hydrophilic and shares amino acid homology with GPIb α and GPIX in the region surrounding the leucine region. The exact role of GPIb β is unknown. GPIb β is larger in endothelial cells than in platelets, and the gene is abundantly expressed in heart and brain.²⁷⁴ The wide distribution of expression among vastly different tissues suggests that GPIb β has more than one role in addition to its function on platelets.

Glycoprotein V. Glycoprotein V (GPV) is an 82-kDa membrane glycoprotein that may function

as a thrombin receptor in the GPIb $\alpha\beta$ /GPV/GPIX complex. Also GPV has been shown to increase the expression of GPIb $\alpha\beta$ /GPIX on heterologous cells.²⁷⁵ It has as many as 15 leucine-rich sequences and a majority of the molecule is on the exofacial side of the plasma membrane.²⁷⁶ This molecule is thought to be the subunit that binds thrombin because the addition of exogenous thrombin cleaves GPV into a 65-kDa soluble form. In addition, GPV knockout mice show an increased aggregation response to thrombin. Thus, it is hypothesized that GPV regulates platelet-associated thrombin activation. Although GPV knockout mice have an increased platelet aggregation response to thrombin, in contrast to in vitro expression studies, they have normal levels of GPIb $\alpha\beta$ /GPIX.²⁷⁷

OTHER PLATELET ANTIGEN SYSTEMS

CD 36: Glycoprotein IV

Platelet glycoprotein IV (GPIV) is also known as the leukocyte differentiation antigen CD36. Along with GPIIb/IIIa, GPIV is the major platelet receptor for thrombospondin. Savill and coworkers²⁷⁸ showed that in the presence of thrombospondin, GPIV participates in the clearance of neutrophils undergoing apoptosis. GPIV also binds collagen, but the physiological significance is uncertain.^{279,280}

Anti-Nak^a antibody defines the GPIV-null phenotype.^{281,282} The antibodies are clinically significant because the specificity was characterized from the serum of an HLA-matched patient who was refractory to platelet transfusions.²⁸³ GPIV deficiency is not uncommon; it is present in 2% to 3% of Japanese, Thais, and Africans, whereas the frequency of the deficiency is less than 0.3% among European Caucasians.²⁸²⁻²⁸⁵ Individuals with GPIV deficiency have no bleeding tendencies or obvious hemostatic defects. However, they risk alloimmunization if transfused with Nak(a+) platelets. GPIV deficiency may be found in some pathological settings. Up to 40% of Japanese with reduced fatty uptake in heart muscle and hereditary hypertrophic cardiomyopathy have a GPIV deficiency.²⁸⁶ Conversely, glucose has been reported to increase the translation efficiency of GPIV, which results in an increase in the expression of GPIV. Macrophages cultured in the presence of high-glucose concentrations showed increased expression of

GPIV. The implications are that increased expression of GPIV provides an environment for increased binding of adhesive ligands and a concomitant increased risk for atherosclerosis in diabetes.²⁸⁷ Lastly, some GPIV polymorphisms are associated with increased risk of malarial infection,²⁸⁸ but none of the polymorphisms have been reported to be antigenic in humans.

GPI-Anchored Protein: CD109 (Gov^{a/b}) HPA-15w System

CD109 is a GPI-linked moiety that is expressed on platelets, some hematopoietic cells, endothelial cells, and T cells.²⁸⁹⁻²⁹¹ CD109 was shown to play a role in T-helper cell function, but its function in other cells is unknown. In some cell types, CD109 is resistant to digestion with phosphatidylinositol-phospholipase C but sensitive to GPI-phospholipase D indicating that the anchor is acylated.²⁹² Lin et al²⁹³ speculated that CD109 may have unique lipid solubility characteristics and may partition into lipid rafts. Lipid rafts represent discrete microdomains on the outer leaflet of the plasma membrane that play a role in activation and signaling. Moreover, a subset of primitive stem cells expresses CD109, suggesting that the molecule is necessary in early cell activity. CD109 is lost on hematopoietic cells after maturation but is detected on activated platelets. CD109 shares homology with α 2 macroglobulin and the C3, C4, and C5 thioester- α -containing proteins. Based on its sequence, CD109 is likely activated by protease digestion and can covalently associate via thioester moieties to adjacent molecules.^{293,294} CD109 has not been given an ISBT designation but expresses the Gov^{a/b} platelet antigen system.²⁹⁴ Antibodies to both phenotypes have been reported.^{292,295,296}

CONCLUDING REMARKS

The presence of some blood group antigens is the result of natural selection (eg, the Duffy GATA-1 mutation occurred on *FYB* and *FYA* independently in *P vivax*-infested areas). Some other amino acid substitutions and silent single nucleotide polymorphism may be represent a "snapshot" of ongoing evolution and are neutral polymorphisms.²⁹⁷ That is to say, they serve no useful purpose and with time will be lost from the random population. Regardless of the existence of various

antigens, RBCs and platelets express a number of structural adhesion and enzyme molecules that vary in their amino acid sequence. We need to examine the interaction of these variant moieties, in the context of their proposed function, with other cells of the vascular and in situ during maturation in both normal and pathologic conditions. Certainly, additional RBC and platelet antigens will be identified in the future, and this work will help us

understand the structure and function of the molecules that express these moieties.

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