Biological Roles of Blood Group Antigens

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Recognition and application of blood group differences on human red cells permitted the development of safe procedures for blood transfusion. Blood group antigens are markers on surface-exposed red cell proteins or the sugar moieties of glycoproteins or glycolipids. Apart from their presumed biological function, some antigens have been identified as receptors for host/parasite interactions. Thus, carbohydrates that determine P antigenicity are the binding receptor for certain strains of pyelonephrititic coliforms. Other pathogenic coliforms bind to the membrane structure that carries the Dr4 antigen. A structure associated with Duffy antigens is the attachment receptor for the parasite of Plasmodium vivax malaria, while Plasmodium falciparum parasites bind to structures associated with membrane glycoporphins.

Structure/function relationships have been established by the finding that lack of Rh protein in red cells of Rhnull phenotype is associated with stomatocytic cell morphology and a hemolytic state. Absence of glycophorin C, and the Gerbich blood group antigens that it carries, is associated with elliptocytic red cells. Absence of Kx antigen protein in the Kell system is associated with the McLeod blood group phenotype, with acanthocytic cell morphology and reduced in vivo survival. McLeod individuals also have late-onset muscular dystrophy and neurological disorders.

Discovery of blood group polymorphisms on human red cells was the major event in the development of safe procedures for clinical transfusion practice. Starting with the ABO system, about 20 independent blood group systems have been recognized, many of them with extensive intra-group antigenic complexity [1]. By virtue of their relative ease of serological detection and their generally straightforward inheritance, blood group studies have been widely utilized in anthropological, genetic, and forensic investigations. In clinical situations, they have been used to monitor in vivo survival of transfused red cells. More recently, they have been used as tools to probe the structure of the red cell membrane. Persistence of blood group genes in evolution suggests that blood group antigens label structures of biological importance. Only recently, however, have associations between the presence of some red cell antigens and red cell function been recognized.

Nearly 600 discrete antigens have been identified [1]. While many of them represent sub-specificities within major blood group systems, others appear to be individual and independent markers. It is probable that all important antigens, from the clinical viewpoint, have now been discovered.

Almost all blood group antigens are inherited characteristics. The only exceptions are the rare acquired A and acquired B antigens and perhaps certain cryptantigens involved in polyagglutination phenomena. Even cryptantigens must be under genetic control, however, for they are concealed below the surface of all red cells. In most cases,

Abbreviations: DAF: decay accelerating factor  Ge: Gerbich (antigen)

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exposure of a cryptantigen by a microbial enzyme converts a latent into an active receptor, which then reacts with an antibody present in all normal human sera.

THE NATURE OF ANTIGENS

A vast literature exists on the serology and formal genetics of the human red cell blood groups. Many of the blood group gene loci have been assigned to specific chromosomes, where they provide important markers. The Xg and Xk systems are X-linked, but all other systems are autosomal. Chromosome 1 carries genes for the Rh, Duffy, Scianna, and Radin systems and is the leader in blood group genetic assignments. Information is growing concerning the biochemical nature of some of the antigenic determinants and the membrane structures that carry them, but relatively little is known of biological functions for these cell-surface-exposed structures.

Understanding of a blood group requires knowledge of its phenotypic complexity and the genetic background from which it arises, the biochemical nature of its antigens and the membrane structure that carries them, and its function in cellular biology. Recognition of a blood group begins with discovery of an antibody. Such an antibody may follow immunization by blood transfusion or pregnancy, and it is thus the blood group serologist who recognizes variation and poses questions that biochemists and cell biologists will subsequently address.

Blood group antigenicity may be a characteristic of a cell-surface-exposed protein or the sugar moiety of a glycoprotein or glycolipid. There is a general correlation between the nature of an antigen and its biological importance. Antigens in which an immunodominant sugar determines specificity, (e.g., A, B, H, Le\(^a\), Sd\(^a\), and so on) do not appear to be vital for normal cell function and survival. Absence of the sugar does not compromise the cell. Conversely, when antigenicity depends upon the nature of a membrane protein, absence of the protein may affect the cell shape and its proper \textit{in vivo} survival. Thus, some null blood types in which a red cell blood group protein is missing are characterized by abnormal cell morphology and a permanent hemolytic state, but null phenotype cells that have missing carbohydrates are normal.

While early studies suggested that most blood group antigens were restricted to erythropoietic tissues, recent reports have described a wider tissue distribution of some of the structures that carry these antigenic markers. Some red cell antigens are also present in solution in plasma and other body fluids (e.g. Lewis, Sd\(^a\), Ch\(^b\)). In most cases, antibodies to these antigens do not cause hemolysis of transfused incompatible red cells, as the antibodies are inhibited by the free plasma antigen. The ability of any blood group antibody to cause hemolytic disease in a newborn infant depends upon whether the reactive antigen is well developed on fetal red cells, whether the antibody is an IgG placenta-passing protein, and whether it has the potential to induce \textit{in vivo} red cell destruction.

BLOOD GROUP ANTIGENS AS BIOLOGICAL RECEPTORS

An important recent development has been recognition of blood group structures as receptors for cell attachment and infection by certain organisms and parasites. Thus, some strains of pathogenic \textit{E. coli} bind to specific structures that are part of P blood group glycosphingolipids on epithelial cells and also on red cells [2]. Binding is important in the initiation of these urinary tract infections and is directly related to bacterial virulence. These strains of pathogenic coliform cannot interact with cells that lack P antigen, the rare p type cells. Filamentous recognition structures on the
coliforms, called pili or fimbriae, interact with a specific disaccharide (galactosyl-galactose) of the P antigen. Binding of pyelonephritic *E. coli* to cells carrying the P antigen is inhibited by a synthetic disaccharide with a structure the same as that of P antigen carbohydrates.

A high-incidence antigen named Dr* appears to be a binding receptor for some other strains of pathogenic coliform [3]. The Dr* antigen has wide distribution on tissue cells, and the capability of these organisms to induce agglutination of Dr(a +) red cells but not of Dr(a −) cells probably reflects a cell-to-cell interaction of biological significance [4]. Further interest has been caused by the finding that Dr* is one of a series of high-incidence red cell antigens that are collectively called “Cromer-related.” Cells of a rare type called INAB lack all of the antigens of this complex [5]. It has been reported that a 70,000 dalton red cell membrane sialoglycoprotein, which carries these antigens, is identical to the decay accelerating factor (DAF) glycoprotein, which is a complement regulatory factor present on many cell types as a surface glycoprotein and also in solution in plasma and other body fluids.

AnWj is the name given to a high-frequency independent antigen, first identified on red cells. The antigen has a wider tissue distribution and has been shown to be a binding receptor on epithelial cells and red cells for *Haemophilus influenzae* [6]. Cells lacking the antigen do not interact with the organism.

Some of the most important receptors for host/parasite interactions involve malaria. Seminal work by Miller and his colleagues first demonstrated that a red cell membrane structure related to the Duffy red blood group is a receptor for attachment of the merozoite of *Plasmodium knowlesi* malaria, a simian parasite that can infect man [7]. By elegant *in vitro* experiments, they demonstrated that malaria merozoites would not attach to, or invade, red cells lacking the major Duffy antigens Fy* and Fy* [i.e., the phenotype Fy(a−b−)]. Further studies established that *Plasmodium vivax*, the parasite causing one type of human malaria, utilizes the same red cell receptor; individuals of Fy(a−b−) type are resistant to this type of malaria [8]. The Fy(a−b−) type is very rare among Caucasians but has a frequency of about 78 percent in African Negroes. The latter finding is presumed to reflect a long period of selective pressure, conferring an advantage on individuals of Fy(a−b−) type in the African environment. *P. falciparum*, the causative parasite of malignant malaria, does not utilize the same receptor for its attachment to red cells [8]. There is evidence that the recognition structure for this parasite is present on the major red cell membrane sialoglycoproteins (glycophorins) [9]. Interaction between *P. falciparum* and red cells does not appear to be a charge-related phenomenon but probably depends on spatial conformation of clusters of oligosaccharides close to the cell membrane forming a specific receptor.

**STRUCTURE/FUNCTION RELATIONSHIPS**

*RHnull*

Red cells of the rare Rh*null* type lack Rh polypeptide and all of the blood group antigens that are products of the Rh gene, which is located on the short arm of chromosome 1. Individuals of Rh*null* type have stomatocytic red cell morphology and shortened *in vivo* red cell survival [10]. The hematological defect is probably a consequence of a cell membrane abnormality caused by lack of Rh protein. The cells have altered membrane permeability in which passive and active transport of Na⁺ and K⁺ are increased [11]. It does not appear, however, that Rh and the Na⁺K⁺ pump
share common structures on the red cell membrane. \( \text{Rhn}_{\text{null}} \) red cells have more \( \text{Na}^+\text{K}^+ \) pumps than normal cells, possibly a compensatory mechanism for the \( \text{K}^+ \) leak [12].

**LEACH PHENOTYPE**

Gerbich (Ge) is a public antigen of almost universal distribution in Caucasian populations. Rare individuals are encountered who are Ge-negative; however, up to 50 percent of the population in some areas of Papua, New Guinea, are Ge-negative [13]. Serum from a Ge-negative person may contain anti-Ge antibody, which creates a major logistic problem in providing compatible blood for transfusion [14].

A particularly interesting variant of the Ge-negative phenotype is called the Leach phenotype. These cells lack the sialoglycoproteins that are collectively called glyco-phorin C and exhibit an elliptocytic red cell shape with reduced in \( \text{vivo} \) survival. The missing sialoglycoproteins, which carry the Gerbich antigenic marker, appear to be important in the organization and function of the red cell membrane [15].

**THE KELL BLOOD GROUP AND MCLEOD SYNDROME**

Kell is one of the major blood group systems of human red cells. Since discovery of the first antibody in 1946, the system has grown steadily in its recognized complexity and presently includes 24 antigens [16]. It is a clinically important system with strong immunogenicity, and active Kell group incompatibility has caused many hemolytic reactions to blood transfusion and numerous cases of severe hemolytic disease in newborn infants.

Among the variant phenotypes in the Kell system, McLeod is one of considerable interest [17]. The McLeod type of red cells react weakly with Kell system antibodies and lack an otherwise ubiquitous antigen named Kx. While Kell phenotypes are inherited as autosomal co-dominant characteristics, inheritance of Kx is through an X-borne gene, named \( \text{Xk} \) [16]. All examples of the McLeod type recognized thus far (\( \geq 60 \)) have been from males, and transmission occurs via a carrier female.

The striking feature of the McLeod blood type has been the clinical syndrome that accompanies it. Abnormalities in these individuals involve erythropoietic, neurologic, and muscular tissue [16]. The red cells have a membrane defect manifested by acanthocytic morphology, changes in the biochemical characteristics of several membrane components, and reduced in \( \text{vivo} \) survival. McLeod subjects show the hematological and biochemical changes expected of a compensated hemolytic state [18]. Muscle changes are manifested by late-onset (usually \( \geq 40 \) years) skeletal muscle wasting, cardiomegaly, and sometimes cardiomyopathy, and elevated levels of serum creatine kinase and carbonic anhydrase. Muscle biopsy has shown changes consistent with a neurogenic myopathy [19]. Neurological changes present as areflexia and slowly progress to choreiform and dystonic movements of some severity. In addition to these changes, there is an association between X-linked chronic granulomatous disease and McLeod syndrome. Some boys with X-linked chronic granulomatous disease also have the McLeod red cell type. The chronic granulomatous disease and \( \text{Xk} \) (McLeod) loci are close together on the X chromosome at Xp21 [20]. In some boys, an X-chromosome deletion spanning both loci has been demonstrated as the cause of the associated condition.

Kell antigens are carried on a 93,000 dalton red cell membrane glycoprotein, but the gene locus has not yet been mapped [21]. Kx antigen is a marker on a 37,000 dalton
membrane protein, encoded by the Xk gene on the X chromosome [22]. Cellular abnormalities correlate with absence of Kx antigen protein.

McLeod Carriers

With recognition that males having the McLeod red cell type develop a significant pathophysiological condition has come a need for accurate identification of female carriers of the gene. The McLeod locus is inactivated by the Lyon phenomenon of X-chromosome inactivation [18]. Somatic cells from female carriers have only one X chromosome expressed, and thus only one functional Xk gene. The phenotypic result is cellular mosaicism in the peripheral blood. One red cell population consists of normal discocytes of common Kell blood group. A second cell population, which derives from an erythropoietic clone in which the mutant Xk gene is expressed, consists of the McLeod type of acanthocytes. Flow cytometric analysis is a sensitive means of demonstrating cellular mosaicism in female carriers of the McLeod gene. Studies using Kell antibodies by flow cytometry on red cells from carriers reveal the double red cell population with high sensitivity [16].

Conclusion

The demands of clinical transfusion practice gave early impetus to blood group studies. Use of blood group antibodies as probes to reveal the structure of the red cell membrane and as markers on receptors involved in host/parasite interactions are important new developments.

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