Alloantigens of Proteins and Glycoproteins in Membranes of Human Red Blood Cells

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INTRODUCTION

Several blood group antigens, i.e. those belonging to the ABH, Lewis, P, I and Sid systems, consist of carbohydrate structures that occur on glycolipids or -proteins in human red blood cell (RBC) membranes. The corresponding gene loci appear to encode the primary structure of glycosyl-transferases (for reviews see Issitt 1985; Salmon et al. 1984). The MNSs system has served as a model for demonstrating that blood group loci may also encode the amino-acid sequences of structural proteins in RBC membranes. Evidence is now accumulating that a number of additional blood group antigens are specifically associated with various different minor (glyco)proteins. It is conceivable that the corresponding blood group loci encode the peptide sequences of these molecules.

RBC membranes contain basically two groups of proteins. The peripheral or extrinsic proteins are loosely attached to the cytoplasmic surface of the lipid bi-layer and can be released by changes of salt concentration or pH value. The major components, spectrin and actin (bands 1, 2 and 5), form a contractile meshwork that is pivotal for maintaining the biconcave shape of RBC. The integral or intrinsic proteins traverse the lipid bi-layer once or several-fold (in the case of the anion channel protein, band 3) with (a) hydrophobic domain(s). Their extracellular portions are usually glycosylated and may carry blood group antigens. The integral proteins form clusters within the bi-layer. There is evidence for specific complexes of different integral components within these clusters that appear to exhibit a bearing for certain antigens. The cytoplasmic portions of some integral components provide attachment sites for a special group of extrinsic molecules, denoted as linking proteins (bands 2.1 and 4.1). The linking proteins mediate reversible binding of spectrin/actin to integral proteins. The integral proteins can be solubilized by detergents such as Triton that purturb the interactions of their hydrophobic domains with lipids. The pellet obtained after extraction is referred to as cytoskeleton. It contains the peripheral proteins, lipids and those integral proteins that are not solubilized because of specific associations with cytoskeletal components. One group of integral proteins, the sialoglycoproteins (SGPs) or glycophorins (GPs), has been studied extensively, since the GPs can be prepared easily by extraction of membranes with organic solvents, due to their high carbohydrate content (up to 60% by weight).

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MNSs SYSTEM

Several aspects of this system whose antigens are located on GP A (MN SGP or α) and GP B (Ss SGP or δ) have been covered by recent reviews (Dahr 1986; Lisowska 1987; Moulds & Dahr 1987; Reid 1986). The 'MNSs locus' is located on chromosome 4, as judged from previous conventional analyses and recent studies with a GP A-cDNA probe (Rahuel et al. 1987). It encodes the amino-acid sequences of two homologous proteins: GP A and GP B. The primary structure (131 residues, res.) and glycosylation sites of GP A were elucidated some time ago. Recent analyses of the cDNA sequence of GP A (Siebert & Fukuda 1986a) have paved the way for studies of genomic DNA that are about to be performed in three laboratories (Siebert & Fukuda 1986b; Rahuel et al. 1987; Huang et al. 1987).

GP A (apparent mol. mass about 35 KDa, about 900,000 copies per RBC) exhibits a hydrophilic, C-terminal, cytoplasmic domain (res. 96-131), a hydrophobic, intramembraneous region (res. 73-95) and an N-terminal, extracellular segment (res. 1-72) that carries about 15 O-glycosidic oligosaccharides (linked to Ser or Thr) and one Asn-linked carbohydrate unit at position (pos.) 26. Amino-acid polymorphisms at the 1st (Ser/Leu) and 5th (Gly/Glu) pos. represent the structural difference between the M and N antigens. Oligosaccharides at the 2nd, 3rd and/or 4th pos. are involved in the epitopes of many anti-M and -N sera. The rare M^C gene encodes an intermediate amino-acid sequence (Ser or Glu at the 1st or 5th pos., respectively) and thus, appears to represent the evolutionary link between the M and N genes. The rare M^g antigen was generated by a Thr → Asn exchange of N-specific GP A. This mutation prevents glycosylation at the 2nd, 3rd and 4th pos. of M^g-specific GP A. Other 'MN variants' (Tm, M_1, Can, Hu, Sext, N^a) appear to be caused by altered oligosaccharides (Dahr 1986; Moulds & Dahr 1987). Some rare MNSs alleles encode amino-acid exchanges at pos. 28 (Mi-I, Mi-II) or 49/52 (Mi-VII, Mi-VIII) that cause an altered glycosylation in these regions (Dahr et al. 1987d). GP A also carries several high-frequency antigens (En^aTS, res. about 26-42; En^aKT, res. 46-56; En^aFR and Wr^b, res. 62-72) (Dahr et al. 1986). GP A forms a specific complex with band 3 that is necessary for optimum incorporation of the molecule into the membrane and that might also exhibit a bearing on the Wr^a/Wr^b polymorphism, the complexity of which is not yet understood at the molecular level (Dahr et al. 1986, 1987b; Unger et al. 1987).

Structural analysis of GP B (72 res., about 11 O-linked oligosaccharides, apparent mol. mass about 24 KDa, about 300,000 copies per RBC) has been completed recently by studies of the protein (Blanchard et al. 1987a) and its cDNA (M. Fukuda pers. commun.). The N-terminal 26 res. of GP B are identical with those of blood group N-specific GP A, thus, providing an explanation for the occurrence of an additional N antigen (denoted as 'N') on GP B. The rare antigens He (exchanges at pos. 1, 4 and 5) and M^v (structure not yet known) are encoded by alleles of 'N'. A Met/Thr polymorphism at pos. 29 of GP B represents the structural difference between the S and s antigens. Studies on Rhnull RBC, which exhibit a decreased level of GP B, have provided evidence that GP B might form a specific complex with Rh-protein(s) that appears to

be necessary for optimum incorporation of GP B into membranes and for optimum expression of the high-frequency antigen U, located within the pos. approx. 34-40 of GP B (Dahr et al. 1987a; Dahr & Moulds 1987). In constrast to GP A, GP B lacks a long cytoplasmic portion. Its intramembraneous region is rather similar to that of GP A (Blanchard et al. 1987a).

Some time ago, it was shown that GP A and/or GP B are absent from (homozygotes), or decreased by about 50% (heterozygotes) in, RBC from individuals exhibiting rare silent alleles (En, u or Mk) at the 'MNSs locus'. Recent studies with cDNA probes have revealed that En(a-) or uu individuals exhibit a deletion of the GP A or GP B gene, respectively (Rahuel et al. 1987; Huang et al. 1987).

Certain rare MNSs alleles represent hybrid gene complexes. Lepore type genes encode hybrid GPs comprising an N-terminal portion from GP A (Mi-V and J.R.: res 1- about 55; English En: res. 1- about 26) and a C-terminal region from GP B (res. approx. 27-72), but no normal GP A and GP B. Typical anti-Lepore type alleles encode GP A, GP B and a hybrid GP comprising an N-terminal region from GP B and a C-terminal portion from GP A. The complete structures of the hybrids associated with the rare St^a and Dantu antigens were elucidated recently (Blanchard et al. 1987b; Dahr et al. 1987e). The St^a-hybrid exhibits the res. 1-28 from GP B and the res. 61-131 from GP A. The Dantu-hybrid possesses the res. 1-39 from s-specific GP B and the res. 72-131 from GP A. There are three varieties of the Dantu allele (Dahr et al. 1987f). The most frequent one, $Dantu^{NE}$, is an atypical anti-Lepore gene complex in that it does not encode GP B. The hybrid in $Dantu^{NE}$ RBC, produced in large quantity, is assumed to suppress the incorporation of GP A into the membrane in a cis and trans manner, via a competitive interaction with band 3 (Dahr et al. 1987b; Unger et al. 1987). As judged from data on the various hybrid GPs, the genes for GP A and GP B appear to be directly adjacent and exhibit the chromosomal alignment GP A-GP B.

GERBICH SYSTEM

The recognition that a rare variety (Leach) of Ge: -1,-2-3 RBC lacks the two minor SGPs GP C (D SGP or ß) and GP D (E SGP or ɣ) as well as the elucidation of the complete structure of GP C by protein and cDNA sequencing have paved the way for rapid analysis of the Ge system at the molecular level (for reviews see Dahr 1986; Reid 1986; Moulds & Dahr 1987). GP C (approx. 100,000 copies per single RBC) comprises 128 res., about 11 O-linked and one Asn-linked carbohydrate units. It exhibits a three-domain structure comparable to that of GP A. Knowledge of GP D occurring only in small quantity (about 20,000 copies per single RBC) is rather limited. The C-terminal portions of GP C and GP D appear to be identical. Both GPs share the Ge3 antigen that is located within the pos. approx. 40-55 of GP C. The Ge2 antigen occurs on the N-terminal portion of GP D, but not on GP C. It is not yet clear whether two separate genes at the Ge locus encode the peptide chains of GP C and GP D. The possibility that GP D represents an abridged version of GP C (res. approx. 20-128), generated by post-translational processing, is also being considered (Le

Van Kim et al. 1987; Dahr et al. 1987c; Reid et al. 1987). Ge: -1, -2,-3 (Gerbich type) and Ge: -1,-2,3 RBC lack GP C and GP D, but contain a novel GP. Individuals exhibiting these phenotypes were found to exhibit a deletion (3 kilobases) within the GP C gene (Le Van Kim et al. 1987). The cytoplasmic portions of GP C and GP D are firmly attached to the linking protein band 4.1. Therefore, both GPs are firmly attached to the cytoskeleton, in contrast to GP A and GP B. GP C and GP D appear to represent the most important attachment sites for band 4.1. Therefore, Ge: -1,-2,-3 RBC (Leach type) are elliptocytic.

Rh SYSTEM

Earlier studies had provided evidence for the involvement of protein (cysteine) and phospholipids in the antigens of this system (for reviews see Issitt 1985; Salmon et al. 1984). The mol. mass of the protein(s) carrying the Rh antigens has been controversial for some time. There is now general agreement that the D, C, c, E and e antigens are located on one or more carbohydrate-free, hydrophobic minor protein(s) exhibiting a mol. mass of about 30 KDa (Moore et al. 1982; Moore 1983; Gahmberg 1983; Ridgwell et al. 1983; Krahmer & Prohaska 1985; Bloy et al. 1987). The molecule(s) could not be detected in Rhnull RBC (regulator and amorphic types) (Gahmberg 1983; Ridgwell et al. 1983). The Rh-proteins are only solubilized to a small extent by Triton extraction (Dahr & Krüger 1983; Gahmberg & Karhi 1984; Ridgwell et al. 1984). It is not yet known whether the molecules are directly attached to a linking protein such as band 4.1 or in an indirect manner via binding to a different integral protein such as GP C. Earlier studies (Moore 1983; Ridgwell et al. 1983; Krahmer & Prohaska 1985) provided some evidence that the D and C, c, E, or e antigens are located on separate molecules. Recent studies have clearly established the occurrence of at least two proteins and provided partial sequence information on the D-active component (Bloy, Blanchard, Dahr, Beyreuther, Cartron unpubl. data).

This author proposes the following hypotheses on the basis of the available serological, genetic and biochemical data: The C/c and E/e polymorphisms are represented by adjacent amino-acid exchanges on one protein. The D antigen is located on a homologous protein that is encoded by an adjacent gene. A deletion of this gene in dd individuals would provide an explanation for the high immunogenicity of the D antigen. The G antigen might represent (an) epitope(s) shared by the D- and C/c-, E/e-molecules. Since the Rh proteins in intact RBC are resistant towards proteinases, it may speculated that they are deeply embedded in the bi-layer. As judged from their mol. masses, they might span the layer several-fold. This author assumes that the Rh proteins exhibit the function to form complexes with certain different integral proteins within the intramembraneous particles (Dahr et al. 1987a) and thus, stabilize the attachment sites for the spectrin-actin meshwork. This hypothesis might provide an explanation for the hemolytic anemia of Rhnull individuals. Apart from alterations of the SsU antigens and a decreased level of GP B (Dahr et al. 1987a), already mentioned above, Rhnull RBC lack additional antigens (LW and Fy5) that are associated with the LW and Duffy systems.

LW SYSTEM

D-negative and D-positive RBC are known to contain different levels of 'D-like' antigens (LW^a, LW^b, LW^{ab}) that are absent from Rhnull and rare LW(a-b-) RBC and encoded by the LW locus. The LW^{ab} antigens were found to be located on a minor glycoprotein containing one or more Asn-linked carbohydrate chains (mol. mass about 43 KDa) that is firmly attached to the cytoskeleton (Moore 1983; Mallison et al. 1986; Anstee 1986). Intrachain disulfide bonds appear to be necessary for optimum antigen expression. This author assumes that the formation of complexes with the Rh proteins represents the prerequisite for incorporation of the LW molecule into the membrane.

DUFFY SYSTEM

The Fy^a and Fy^b antigens are located on a minor protein exhibiting a mol. mass of about 45 KDa as revealed by immunoprecipitation or -blotting (Moore et al. 1982; Moore 1983; Hadley et al. 1984; Lisowska et al. 1983; Dahr & Krüger 1983; Anstee 1986). Glycosidase digestions revealed that the molecule carries one or more Asn-linked carbohydrate chains. The Duffy-protein is not attached to the cytoskeleton, since it can be solubilized completely by a low Triton concentration. It contains cysteine, and therefore, may be separated from other components by affinity chromatography on Thiopropyl-Sepharose (Lisowska et al. 1983; Dahr & Krüger 1983). The possibility that the Fy5 antigen is represented by a complex involving Rh- and Duffy-molecules has already been mentioned above.

LUTHERAN SYSTEM

The protein(s) carrying the Lu^a and Lu^b antigens was found to exhibit properties similar to those of the Duffy-protein: It could be solubilized by a low Triton concentration and was bound by Thiopropyl-Sepahrose. However, the Fy and Lu antigens could be separated by ion exchange chromatography of Triton extracts (Dahr & Krüger 1983). Recent immunoblotting studies with a monoclonal anti-Lub showed that the molecule carries one or more Asn-linked carbohydrate chain(s) and exhibits a mol. mass of about 80 KDa (Anstee 1986; Parsons et al. 1987). It is not at all clear whether the Lu locus encodes the primary structure of the 80 KDa component. Udden et al. (1987) have presented evidence that Lu(a-b-) RBC of the In(Lu) type exhibit a carbohydrate alteration. Telen et al. (1984) have suggested that In(Lu) which represents a dominant suppressor gene, not linked to the Lu locus, regulates the expression of the 80 KDa component.

KELL SYSTEM

Redman et al. (1984) and Wallas et al. (1986) have shown that the K1 (K), K2 (k), K7 and K22 antigens are located on a minor glycoprotein possessing a mol. mass of about 93 KDa. This component exhibits at least one intra-chain disulfide bond that ap-

pears to account for the known destruction of these antigens by reducing agents. The 93 KDa protein was found to form a disulfide complex with a 32 KDa component. It is tempting to speculate that the latter molecule is encoded by the Kx locus. The Kell antigens are firmly attached to the cytoskeleton (Dahr & Krüger 1983). Since Ge: -1,-2,-3 RBC are known to exhibit a depression of the Kell antigens, the 93 KDa might be attached to GP C rather than to a linking protein. Recently (Marsh et al. 1987), isolation of the 93 KDa component has been used for demonstrating that a novel low-frequency antigen (K23) belongs to the Kell system.

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