Biochemistry of Red Blood Cell Antigens: Carbohydrate Antigens

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Blood groups normally defined by serological properties of erythrocytes are based on specific stereochemical configurations of the red cell membrane constituents. Since lipids in general are poor antigens, it must be expected that proteins and carbohydrate structures act as carriers of the antigenic determinants.

The antigens of the systems MNS and Rhesus are the most important within the protein-dependent blood groups. The determinants of the systems ABO(H), Lewis and P, the Ii-antigens, the so-called T-antigens and the characters Sid and Cad are based on oligosaccharide structures.

The first carbohydrate-dependent blood group systems to be investigated chemically were ABO(H) and Lewis. Thanks to the fact that the antigens of these systems are not confined to the red cell membrane but, rather, that great amounts of ABH and Lewis active glycoproteins are found in various body secretions (e.g. saliva, gastric juice and ovarian cyst fluid), it has been possible to isolate blood group active substances in quantities sufficient to determine the structures of the antigenic determinants.

According to the investigations of Morgan (Lister Institute of Preventive Medicine, London) and Kabat (Columbia University, New York), the carbohydrate chains carrying the ABO(H) and Lewis characteristics are built of four monosaccharides: D-galactose (Gal), L-fucose (Fuc), N-acetyl-D-galactosamine (GalNAc) and N-acetyl-D-glucosamine (GlcNAc). The basic structure of the A, B, and H determinants is represented by two types of carbohydrate chain endings - the terminus **Gal**ß1-3**GlcNAc**- ("type 1 chain") with no known serological specificity, and the terminus **Gal**ß1-4**GlcNAc**- ("type 2 chain") which is part of the I and i determinants and is responsible for the cross-reactivity of blood group substances with anti-type-14 pneumococcus sera. The chemical structures of the ABO(H) and Lewis serological determinants show that these blood groups are characterized by only one or two monosaccharide units:
An α1-2 fucose on the terminal galactose residue of a type 1 or type 2 chain is characteristic for blood group H activity. An H-specific structure with additional α1-3 N-acetylgalactosamine or α1-3 galactose shows A or B specificity, respectively. A

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Fig. 1: The structures of the determinants of the blood group systems ABO(H) and Lewis.

Basic chain type 1: **Gal**ß1-3**GlcNAc**ß1-3**Gal**-

Basic chain type 2: **Gal**ß1-4**GlcNAc**ß1-3**Gal**-

H determinant: **Gal**ß1-3/4**GlcNAc**ß1-3**Gal**-
α1-2
Fuc

A determinant: **GalNAc**α1-3**Gal**ß1-3/4**GlcNAc**ß1-3**Gal**-
α1-2
Fuc

B determinant: **Gal**α1-3**Gal**ß1-3/4**GlcNAc**ß1-3**Gal**-
α1-2
Fuc

Le^a determinant: **Gal**ß1-3**GlcNAc**ß1-3**Gal**-
α1-4
Fuc

Le^b determinant: **Gal**ß1-3**GlcNAc**ß1-3**Gal**-
α1-2 α1-4
Fuc **Fuc**

type 1 chain ending with an α1-4 fucosyl group on the subterminal N-acetylglucosamine residue is responsible for the Le^a character, while in the case of an additional blood group H determinant α1-2 fucose the specificity is changed to Le^b (see Fig. 1). The serological determinant groups are attached to oligosaccaride units of various lengths which in turn are bound to sphingosine (→glycosphingolipids) or protein (→ glycoproteins); in the cell membranes glycosphingolipids as well as glycoproteins are present, while in secretions only blood group active glycoproteins occur. Group-specific oligosaccharides with analogous terminal structures have been isolated from milk

and urine.

The blood group phenotype expressed on the basis of genetic information is essentially dependent on the mechanism by which the blood group substances are formed. In contrast to proteins, which are synthesized in a continuing sequence translated from an mRNA, the oligosaccharide chains of the glycoconjugates are assembled stepwise by highly specific glycosyl transferases. The monosaccharide units are joined together according to a sequential mechanism, in which the product of each single step represents the acceptor substrate for the transfer step following it in the sequence, with sugar nucleotides (UDP-Gal, UDP-GalNAc, UDP-GlcNAc and GDP-Fuc) acting as monosaccharide donors.

It is generally accepted that one gene codes for one glycosyl transferase; and therefore a whole set of transferases, and thus genes, must be assumed for the biosynthesis of the whole carbohydrate chain. For the encoding of the enzymes responsible for the transfer of the blood group ABO(H) and Lewis determinant monosaccharide residues, three gene systems have been postulated - A/B/O, H/h and Le/le; the ability of the individual to secrete blood group ABH active substances in mucous tissues is controlled by the gene system Se/se.

According to this scheme the H gene codes for an α1-2 fucosyl transferase, the Lewis gene for an α1-4 fucosyl transferase, and the genes A and B for α1-3 N-acetylgalactosaminyl or α1-3 galactosyl transferases, respectively. The genes O, h, and le are considered silent alleles which do not encode enzymatically active glycosyl transferases. In cases where these alleles are present in double dose, i.e. on both chromosomes, the individual is not able to express A/B, H, or Lewis properties, resp.

The blood group gene dependent glycosyl transferases occur in serum and various tissues and have been investigated by the groups of Watkins (Lister Institute of Preventive Medicine, London), Ginsburg (National Institutes of Health, Bethesda, MD, USA), Schachter (University of Toronto, Ontario, Canada), and by our institute. The results of these investigations clearly show that the occurrence and the distribution of the enzymes in different organ systems is in close relationship to the respective genotype of the individual:

The A gene-dependent α1-3 N-acetylgalactosaminyl transferase is found in all individuals carrying the allele A, i.e. A and AB subjects; similarly, the B-enzyme - the α1-3 galactosyl transferase - occurs in all individuals carrying the allele B, i.e. B and AB subjects. In blood group O subjects with the silent allele O in double dose, no transferase is present to transform the H substance, and thus the occurrence of large amounts of the unchanged precursor substance is characteristic for blood group O individuals.

Human blood group H substance is produced from its precursor by the action of an α1-2 fucosyl transferase. A transferase with

this specificity is found in all sera except those of 'Bombay' (O_h) - individuals; in secretory tissue the enzyme is present only in secretors but is absent in non-secretors. In the classical model the secretor gene (Se) that determines the secretor status is considered a regulatory gene controlling the expression of H gene-specified fucosyl transferase in the secretory tissue. According to recent biochemical evidence, however, H and Se are both structural genes encoding two α1-2 fucosyl transferases with different characteristics: the secretor-transferase prefers to attach the α1-2 fucosyl residues to type 1 chains, and the H-transferase prefers type 2 chains.

The occurrence and distribution of the α1-2 fucosyl transferase show that the H gene-specified enzyme ("H transferase") is expressed in haematopoietic tissues and serum, and is absent in secretory tissues and secretory fluids, while the Se gene-specified enzyme ("secretor transferase"), on the other hand is expressed in secretory tissues such as gastric mucosa and submaxillary glands, and in secretory fluids. It is absent in haematopoietic tissues.

In non-secretors homozygous for the silent allele se, the "secretor-enzyme", the α1-2 fucosyl transferase, is absent. Since the A and B transferases are dependent on the H determinant as the precursor substrate, no ABH determinants can be formed in secretory tissue. The ABH antigens on the erythrocyte surface are unaffected, as they are synthesized by the action of the H-transferase.

The absence of the α1-2 fucosyltransferase in 'Bombay' serum is in full agreement with the earlier assumption that 'Bombay' individuals are homozygous for the silent allele h, which is not able to code for an enzymatically active α1-2 fucosyl transferase. In this case no A, B, or H antigens can be synthesized on the erythrocyte precursor cells. Since ABH active substances are also absent in body secretions, 'Bombay' individuals are probably ABH non-secretors.

The Lewis blood group is genetically independent of the ABH system; the appearance of the different Lewis characters, however, is closely connected to the secretor status of the individual: Lewis-positive individuals who are ABH secretors show Le^b specificity on red cells and in body secretions, while in non-secretors only Le^a activity is found.

Earlier investigations clearly show that the Lewis blood group substances of the erythrocytes are not synthesized in the red cells themselves but are absorbed from the plasma. Considering the fact that the Lewis phenotype is dependent on the secretor status, it can thus be concluded that the Lewis blood group substances are synthesized in a system influenced by the secretor gene.

The distribution of the α1-4 fucosyl transferase - the enzyme encoded by the Lewis gene - fully supports this assumption: the enzyme is found only in secretory tissue of Lewis-positive

individuals; it is absent, however, in serum. The occurrence of the fucosyltransferases ("Secretor transferase" and "Lewis transferase") in mucous tissue parallels the Lewis blood group phenotype of the individual: in secretors where both enzymes - the α1-2 and α1-4 fucosyltransferase - are present, Le^b substance is synthesized, while in non-secretors, where the secretor gene dependent α1-2 fucosyltransferase is absent, only Le^a substance can be formed.

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