Cell membrane receptors for serological reagents

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Many useful genetic markers are cell surface structures. Furthermore, the cell surface is intimately involved in cellular immunity, and often in humoral immunity. It is therefore important, both in genetics and immunogenetics, to understand cell surface structure.

Although the cell membrane has been actively studied for many years, progress in this field is understandably slow. It is generally agreed that the cell membrane is fundamentally a phospholipid bilayer with inner and outer hydrophilic areas and a central hydrophobic region. Besides lipids, the membrane contains proteins and carbohydrates; the carbohydrates occur as glycolipids or glycoproteins.

Whereas the protein and lipid portions of the cell membrane occupy the plane of the membrane, the carbohydrate chains of the glycolipid and glycoprotein components of the cell membrane project outwards, so that their N-terminal ends are exposed above the membrane surface. Recent work, chiefly by Singer and Nicolson (1972) and Nicolson (1973), on the structural organization of membranes, in terms of the thermodynamics of macromolecular systems, has led to the conclusion that the cell membrane is a mosaic of alternating globular proteins and phospholipid units, in which the phospholipid units represent the bricks and the globular protein the mortar (Fig. 1). The mosaic structure is dynamic rather than static, with considerable lateral movement of the phospholipid and protein components. Different membrane components move laterally in the plane of the membrane at different rates, depending on restraints applied from outside or inside the cell. The movement may account for certain apparent changes on the cell surface, such as the appearance of 'new' HL-A specificities on cultured cells (Mackintosh, Hardy, and Aviet, 1971), or for changes observed after treatment of intact cells by

enzymes. Indeed, it has been suggested (Singer and Nicolson, 1972) that after proteolysis or malignant transformation, agglutinin-binding sites diffuse and aggregate in clusters (Fig. 2). This might explain, for example, the very strong agglutination of papain-treated A₂ cells by the virtually A₁-specific agglutinin obtained from the seeds of *Dolichos biflorus*. Although this lateral movement may sometimes affect the 'display' of surface components, molecular orientation is generally maintained.

Cell surface carbohydrates

We know more about the structure of erythrocytes than about any other cells, and more about those antigens which are carbohydrate than those which are lipid, protein, or lipoprotein.

There are only seven monosaccharide components of the human red cell membrane (Winzler, 1969): galactose, mannose, fucose, glucose, acetylgalactosamine, acetylglucosamine and N-acetylneuraminic (sialic) acid. All human erythrocyte membrane carbohydrate chains are therefore combinations of these seven sugars, and differ from one another in the serial arrangement of the sugar components, and the nature of the chemical linkages between them. There are therefore structural similarities in receptor sites for various serological reagents, so that it is not surprising that they crossreact; what is surprising therefore is that many reagents are very highly specific.

The ABH and Lewis systems

The ABH and Lewis blood group antigenic determinants are the carbohydrate moieties of glycoproteins or glycolipids. Part of the work which led to the elucidation of ABH and Lewis structure was done, not on red cells, but on blood group specific substances present in soluble form in certain body fluids, eg, saliva of secretors, pseudomucinous

FIG. 1. The fluid mosaic model of the cell membrane; schematic three-dimensional and cross-sectional views, showing the phospholipid bilayer and the globular integral protein (solid bodies with stippled surfaces). (According to Fig. 3 in Singer, S. J. and Nicolson, G. L., 'The fluid mosaic model of the structure of cell membranes', *Science*, **175**, 18 February 1972. Copyright 1972 by the American Association for the Advancement of Science. Reproduced with the permission of authors and the editor, *Science*.)

ovarian cyst fluids (Kabat, 1956; Morgan and Watkins, 1959; 1969).

The earliest indication that a single component sugar (the 'immunodominant' sugar) is more closely involved in serological specificity than any other was obtained by means of agglutination-inhibition tests with simple sugars (Morgan and Watkins, 1953). This approach was based on the observations of Landsteiner (1920) that low molecular weight substances with structures identical with, or closely similar to, the immunologically determinant group of a complex antigen combine with the agglutinating reagent, and completely inhibit its reaction with the corresponding antigenic determinant. The agglutinating reagents first used for this purpose by Morgan and Watkins (1953) were those specific for small configurations, such as the anti-H agglutinins present in the sera of certain eels, or in *Lotus tetragonolobus* seeds, and which are therefore inhibited by simple sugars.

The capacity of L-fucose, a component sugar of

H-substance, to inhibit the anti-H agglutinins of eel serum or *Lotus tetragonolobus* shows that this sugar is an important determinant of H-specificity. Similar studies show that N-acetyl-D-galactosamine is an important structural determinant of A- and D-galactose of B-specificity. These observations have been confirmed by other methods.

Biosynthesis of ABH and Lewis

The primary product of a blood group gene is an enzyme (glycosyltransferase) which attaches the characteristic end sugar to a preformed substrate (Morgan and Watkins, 1969), presumably created by the sequential action of other transferases.

The precursor substrate for the ABH and Lewis substances consists of two carbohydrate chains (Table IA). The product of the *H* gene, a fucosyltransferase, adds L-fucose to both chains (Table IB). The product of the *A* gene, an N-acetyl-D-galactosaminyltransferase, adds N-acetyl-D-galactosamine

FIG. 2. Two mechanisms to explain the enhanced action of lectins on malignant transformed or protease-treated erythrocytes: (A) removal of steric hindrance; (B) clustering of receptor sites. (According to Fig. 7 in Singer, S. J. and Nicolson, G. L., 'The fluid mosaic model of the structure of cell membranes', *Science*, 175, 18 February 1972. Copyright 1972 by the American Association for the Advancement of Science. Reproduced with the permission of authors and the editor, *Science*.)

to the H-chains (Table IC). Similarly the product of the *B* gene, a D-galactosyltransferase, adds D-galactose to the H-chain (Table ID).

The product, *Le*^a, of the *L* (Lewis) gene, which is a different fucosyltransferase to the product of the *H* gene, attaches L-fucose to the penultimate sugar only of chain 1 (Table IE). The ABH and Lewis genes, which are carried on different chromosomes, act on the same substrate, and therefore interact in their phenotype expression. In the presence of both *H* and *L* genes, the precursor substance is converted as shown in Table IF. It will be seen that the *Le*^b character, which at one time was thought to be the product of a gene allelic to the *Le*^a (*L*) gene, is really an interaction product of the *H* and *L* genes. Indeed, in the presence of *H* and *L* genes, one of the precursor chains is converted into *Le*^b and the other into H. It is not surprising therefore that crossreactivity between H and *Le*^b is frequently observed.

Glycosyltransferases

The glycosyltransferases involved in ABH and Lewis biosynthesis are present in various tissues and body fluids (Race and Watkins, 1972). They are present in soluble form in serum. The origin and function of glycosyltransferases are unknown. They can be shown to act *in vitro* by incubating O cells in a mixture containing α -N-acetyl-D-galactosamyltransferase and nucleotide-bound N-acetyl-D-

galactosamine. The O cells then develop the A character. Similar results are obtained with the B-enzyme and the B-sugar. That transferases do not act on erythrocytes *in vivo* is evident from observations made on blood group chimæras in which, for example, a mixture of group O and A₁ erythrocytes persists throughout life. Race and Watkins (1972) state that the co-existence in chimæras of two independent cell populations indicates that the final step in the formation of A- or B-active sites on the erythrocyte surface is not mediated in the external environment of the cells.

The biosynthetic pathways established by the work of Morgan and Watkins (1959; 1969) and of Kabat (1956) have firm experimental support and are generally accepted. Some alternative hypotheses have been proposed, notably by Weiner, Socha, and Gordon (1972), who propose that H is not a precursor of A and B, and that the *H*, *A*, and *B* genes compete with one another for a common precursor substrate.

Red cells

Since information derived from the study of blood group specific substances in body fluids does not necessarily apply to red blood cells, the cells themselves are now being actively studied. It has been shown that whereas in body fluids, the ABH blood group specific substances are glycoproteins, they occur in the red cells as glycolipids and glycoproteins. They occur as glycolipids in the red cells of all persons and also as glycoproteins on the red cells of secretors of blood group specific substances (Gardas and Kościelak, 1971). One of the most valuable and perhaps the least harsh of the methods of investigating the fine structure of the red cell surface is to study the reactions of plant seed lectins of known chemical specificity before and after treatment of red cells with various enzymes.

Lectins

Indeed, the current 'explosion' in the application of lectins to the study of cell surface structure has greatly exceeded the expectations of earlier workers in this field. The subject has been reviewed by Bird (1959) and Boyd (1963). Lectins are now being extensively and usefully applied to structural studies not only of red blood cells but also of white blood cells, tumour cells, bacteria and viruses. A recent number (vol. 234) of the *Annals of the New York Academy of Sciences* is devoted to a symposium held on this subject. Lectins are particularly useful in studies of cell membrane structure because they combine with single sugar

TABLE I
BIOSYNTHESIS OF ABH-LEWIS AND (?) I SUBSTANCES

	Designation of Chain	Gene	Structure of Gene Product	Specificity
A	1	?	$\beta\text{-Gal} \xrightarrow{1 \rightarrow 3} \beta\text{-GNac} \longrightarrow$	—
	2		$\beta\text{-Gal} \xrightarrow{1 \rightarrow 4} \beta\text{-GNac} \longrightarrow$	May be related to I-specificity
B	1	<i>H</i>	$\beta\text{-Gal} \xrightarrow{1 \rightarrow 3} \beta\text{-GNac} \longrightarrow$ $\uparrow 1 \rightarrow 2$ $\alpha\text{-Fuc}$	H
	2		$\beta\text{-Gal} \xrightarrow{1 \rightarrow 4} \beta\text{-GNac} \longrightarrow$ $\uparrow 1 \rightarrow 2$ $\alpha\text{-Fuc}$	H
C	1	<i>H and A</i>	$\alpha\text{-GalNAc} \xrightarrow{1 \rightarrow 3} \beta\text{-Gal} \xrightarrow{1 \rightarrow 3} \beta\text{-GNac} \longrightarrow$ $\uparrow 1 \rightarrow 2$ $\alpha\text{-Fuc}$	A
	2		$\alpha\text{-GalNAc} \xrightarrow{1 \rightarrow 3} \beta\text{-Gal} \xrightarrow{1 \rightarrow 4} \beta\text{-GNac} \longrightarrow$ $\uparrow 1 \rightarrow 2$ $\alpha\text{-Fuc}$	A
D	1	<i>H and B</i>	$\alpha\text{-Gal} \xrightarrow{1 \rightarrow 3} \beta\text{-Gal} \xrightarrow{1 \rightarrow 3} \beta\text{-GNac} \longrightarrow$ $\uparrow 1 \rightarrow 2$ $\alpha\text{-Fuc}$	B
	2		$\alpha\text{-Gal} \xrightarrow{1 \rightarrow 3} \beta\text{-Gal} \xrightarrow{1 \rightarrow 4} \beta\text{-GNac} \longrightarrow$ $\uparrow 1 \rightarrow 2$ $\alpha\text{-Fuc}$	B
E	1	<i>Le</i>	$\beta\text{-Gal} \xrightarrow{1 \rightarrow 3} \beta\text{-GNac} \longrightarrow$ $\uparrow 1 \rightarrow 4$ $\alpha\text{-Fuc}$	Le ^a
	2		$\beta\text{-Gal} \xrightarrow{1 \rightarrow 4} \beta\text{-GNac}$	
F	1	<i>H and Le</i>	$\beta\text{-Gal} \xrightarrow{1 \rightarrow 3} \beta\text{-GNac} \longrightarrow$ $\uparrow 1 \rightarrow 2$ $\alpha\text{-Fuc}$	Le ^b
	2		$\beta\text{-Gal} \xrightarrow{1 \rightarrow 4} \beta\text{-GNac} \longrightarrow$ $\uparrow 1 \rightarrow 2$ $\alpha\text{-Fuc}$	H

Fuc = fucose; Gal = galactose; GNac = N-acetyl-D-glucosamine; GalNAc = N-acetylgalactosamine.

units, usually in terminal position in the carbohydrate chains. The specificity of some lectins is given in Table II. The mere presence of the reactive sugar is not the sole determinant of lectin specificity. Other factors which influence lectin activity include the nature of the next-to-terminal structure, the type of chemical linkage to this structure, the amount of steric hindrance provided by neighbouring structures, and the number and distribution of receptor sites.

The 'Bombay' blood group

In the rare 'Bombay' blood group (H-negative, genotype *hh*), the H-forming, α -fucosyltransferase is

absent, so that the precursor substrate is not converted into H. Therefore, even if *A* or *B* genes are present, they cannot act, because their normal substrate, H-substance, is absent. 'Bombay' blood therefore has no ABH antigens.

The MN system

Much work on the genetics of the MN blood group system has been based on the assumption that the M and N characters are the products of allelic *M* and *N* genes. Recent studies of the biosynthesis of antigens of the MN system show that this assumption is wrong. It now seems certain (Springer and Huprikar, 1972) that N is a basal substance, analo-

TABLE II
THE SPECIFICITY OF SOME LECTINS

Source	Sugar Specificity	Blood Group or Bacterial Specificity
<i>Lotus tetragonolobus</i>	L-fucose	H
<i>Dolichos biflorus</i>	N-acetyl-D-galactosamine	A Cad Tn <i>Streptococcus C</i> <i>Salmonella riogrande</i>
<i>Fomes fomentarius</i>	α -D-galactose	B P
<i>Vicia graminea</i>	β -D-galactose	N
<i>Arachis hypogaea</i>	β -D-galactose	T
<i>Salvia sclarea</i>	N-acetyl-D-galactosamine	Tn
<i>Salvia horminum</i> *	N-acetyl-D-galactosamine	Tn
<i>Salvia horminum</i> *	N-acetyl-D-galactosamine	Cad

* *Salvia horminum* contains separable anti-Tn and anti-Cad agglutinins.

gous to H in the ABH system, and that the biosynthetic pathway for M and N is probably as shown below:

The addition of β -linked D-galactose to the precursor material results in the formation of N_{Vg} , the receptor which combines with the anti-N agglutinin present in the seeds of *Vicia graminea*. Subsequent addition of sialic acid results in the formation of N. Further addition of sialic acid converts N to M.

Human blood group N specificity (Springer and Huprikar, 1972) is due to branched structures which end in both α -linked sialic acid and β -linked D-galactose. M differs from N only in that sialic acid covers the terminal β -D-galactose of N. The *Vicia graminea* anti-N lectin combines with the β -galactosyl residue and therefore does not ordinarily agglutinate M cells. Human and rabbit anti-N react with the sialic acid moiety. In most persons, the conversion of N to M is not complete, so that anti-N sera tend to crossreact with M cells. According to Dawson (1969), the converse is true of tissue cells: anti-M crossreacts with N cells. If red cells are exposed to neuraminidase, an enzyme which specifically removes sialic acid, M and N activity as demonstrated by human or rabbit anti-M or anti-N is lost, but activity against *Vicia graminea* anti-N is enhanced. In view of these observations, we must now think of the M-character as being produced by an M gene allelic to an m gene, which is an amorph. We have as yet little knowledge of the biosynthesis

of the S, s, U, u characters, or of the many M and N variants, eg, M^k , M^s , N_2 (Metaxas, Metaxas-Bühler, and Ikin, 1968) or of the 'satellite' antigens of this system, eg, He, Hu, Vw (Race and Sanger, 1968).

Sialic acid

N-acetylneuraminic acid, better known as sialic acid, is an important red cell membrane component. It contributes largely to the negative charge of the cell surface and therefore to the maintenance of the normal separate state of red cells. It forms an important part of various human erythrocyte receptors, eg, M, N, Pr_1 . Some red cells are sialic acid-deficient; the deficiency may be acquired, as in T- or Tn-polyagglutinable erythrocytes, or inherited as in $En(a-)$, M^k or M^s cells (Bird, 1971).

Tn-polyagglutination

Although Tn-polyagglutination is an acquired condition, it is of some interest in genetics because it is believed to arise from somatic mutation. Tn-polyagglutination is comparatively rare and occurs in previously normal persons; once it appears it persists. No example of its spontaneous disappearance has yet been reported. However, the condition could theoretically disappear by elimination of the mutant clone. Two red cell populations are present, Tn and non-Tn. It presents as a 'mixed-field' condition, because only the Tn cells are agglutinated by anti-Tn present in most human adult sera or extracted from certain plant seeds. Indeed, plant seed reagents have proved very useful in the elucidation of erythrocyte polyagglutination, and in studies of the chemical structure of the various receptors responsible for polyagglutinable erythrocytes (Bird, 1971; Bird and Wingham, 1974a).

Tn-cells are strongly agglutinated by the lectins from *Dolichos biflorus* and other seeds which are specific for α -linked N-acetyl-D-galactosamine (see Table I). Recent studies by Dahr, Uhlenbruck, and Bird (1974) have shown that the Tn receptor is a cryptantigen which probably has the structure:

The M, N and N_{Vg} receptors of Tn-erythrocytes are impaired (Sturgeon *et al*, 1973; Bird and Wingham, 1974b).

$En(a-)$, M^k , and M^s

Lack of a very common red cell structure, En^a , is a rare recessive character, $En(a-)$, first described by Darnborough, Dunsford, and Wallace (1969). $En(a-)$ red cells have only about one third of the normal content of sialic acid. Their M and N

TABLE III
CLASSIFICATION OF CAD/Sd^a GROUPS*

	Reactions with <i>Dolichos biflorus</i>	Mixed- field	Polyagglutination
CAD 1	+++	No	Yes
CAD 2	+++	Yes	No
CAD 3	++	Yes	No
Strong Sd ^a	+	Yes	No
Sd ^a	—	—	No

* There may be some overlap between the various categories.

antigens are weak, as are their receptors for the *Maclura aurantiaca* lectin, and the cells are agglutinated when suspended in physiological saline solution by 'incomplete' antibody (Darnborough *et al*, 1969; Furuhielm *et al*, 1969; Bird and Wingham, 1973). En(a—) persons can produce immune anti-En^a antibodies.

Furuhielm *et al* (1969) suspect that the condition is caused by the lack of an enzyme needed for the building of a normal cell surface (the term En stands for envelope) and that the En(a—) condition can be thought of as 'an inborn error of metabolism'.

Darnborough *et al* (1969) postulated the involvement of two codominant genes, En^a and En^b. The term En is generally preferred to En^b. There are therefore three genotypes:

En^a En^a
En^a En
En En

The cells of heterozygotes react in a similar manner to those of homozygotes, but to a lesser extent.

The M^k gene gives rise to none of the antigens of the MN or Ss series; the presence of its product is manifest in the heterozygous state by single dose reactions with anti-M or anti-N, and by indications of abnormality of the red cell surface, ie, sialic acid deficiency, which causes the cells to be agglutinated by various 'incomplete' antibodies. There is probably no anti-M^k antibody as such. The M^k gene is probably an amorph, analogous to the O gene of the ABO system, or the d gene, if any, of the Rhesus system. It might be a 'built-in' inhibitor gene at the MNSs locus. The subject has been extensively treated by Metaxas, Metaxas-Bühler, and Romanski (1971). The reactions of M^k cells are similar to those of En^a En heterozygotes.

M^s cells react similarly to M^k (Nordling *et al*, 1969). Anti-M^s is a relatively common antibody. En(a—), M^k, and M^s are however different to one another (Nordling *et al*, 1969).

En(a—), M^k, and M^s cells have so far shown no evidence of shortened survival.

P

P₁-substance is present in hydatid cyst fluid. Morgan, Watkins, and Cory (1972) have isolated a P₁-active glycoprotein from this material. The immunodominant sugar in P₁-specificity is an α-D-galactosyl residue.

I

Studies by Feizi *et al* (1971a and b) on soluble I-substance obtained from milk showed that I-specificity is related to the ABH and Lewis glycoproteins. These workers concluded that the synthesis of I-substance precedes that of ABH and Lewis. Earlier studies with enzymes which destroy I-substance had suggested that β-N-acetyl-D-glucosamine and β-D-galactose residues are involved in I-specificity.

Work on the Ii system is greatly bedevilled by heterogeneity of both antigens and antibodies. Feizi *et al* (1971b) have shown that the specificity of one anti-I serum is probably directed towards the structure β-D-Gal-(→4)→β-D-GNAc→. This structure is present in Chain 2 of the ABH and Lewis precursor substance (see Table IA).

Cad/Sd^a

Cad is a rare inherited blood group character first reported by Cazal *et al* (1968). Cad cells are agglutinated, irrespective of ABO blood group, by *Dolichos biflorus* seed extract. Cad is inherited independently of ABH. Subsequently, various grades of Cad-positivity were described (Cazal, Monis, and Bizot, 1971).

Sanger *et al* (1971) made the interesting observation that Cad is probably a strong form of the Sid or Sd^a antigen. An attempted classification of the various Cad/Sd^a grades is shown in Table III. This classification is arbitrary; there may be some overlap between one category and the next.

The capacity of the *Dolichos biflorus* agglutinin strongly to agglutinate Cad cells shows that α-N-acetyl-D-galactosamine is also the chief structural determinant of Cad specificity. Thus, there is a resemblance between the A, Tn, and Cad receptors. It is therefore remarkable that there are some plant seed reagents which can clearly distinguish these three receptors (Bird and Wingham, 1974a).

Rhesus

Comparatively little is known of the structure of the Rhesus antigens. Studies by Green (1968a and

b) suggest that they are probably lipoproteins. Extraction of the red cell membrane lipid with *n*-butanol causes loss of Rhesus activity which can be restored by subsequent exposure of the membrane to the extract. Inactivation of Rh determinants by heat or treatment with some sulphhydryl compounds indicates that the protein moiety is an essential part of Rh antigen structure.

An interesting genetic condition is the Rh_{null} state, in which the red cells are devoid of the products of any of the Rhesus genes. The condition arises either when a person is homozygous for a rare recessive gene X^0r , allelic to a common regulator gene X^1r , or when a person is homozygous for a rare silent or amorphic gene R_{null} , within the Rhesus locus. The regulator type can be recognized when a parent or child has normal Rhesus antigens. In the amorphic type, family studies may show, for example, an apparently CDe/CDe parent with an apparently cDe/cDe child. The explanation, of course, is that the parent is CDe/— — and the child — —/cDe.

The absence of specific lipoprotein sites apparently has an adverse effect on the red cell membrane, so that in the Rh_{null} condition there is a mild haemolytic anaemia (Schmidt and Holland, 1971), and the sodium pump has to work twice as hard as in normal persons (Levine, 1974).

The haemolytic anaemia is associated with shortened red cell survival, stomatocytosis, increased red cell fragility, mild spherocytosis, and a high reticulocyte count. Schmidt and Holland (1971) gave the name 'Rh_{null} disease' to this condition; the name is not satisfactory because a similar condition occurs in Rh_{mod} , in which Rh antigens are present but are weaker than normal (Chown *et al*, 1972).

In the 'Bombay' blood group, however, red cell survival is normal. Levine *et al* (1973) attribute the difference to the fact that the defect in the Rh_{null} condition involves sites in the plane of the membrane, whereas that in the 'Bombay' blood group involves oligosaccharide chains, which project above the plane of the cell surface.

Rh and LW

The gene *LW* responsible for LW, a character which very closely resembles the Rhesus antigen D, is inherited independently of the Rhesus genes (Tippett, 1963). According to Giblett (1969), Rh may represent an intermediate step in the biosynthesis of LW (Fig. 3). The fact that all Rh_{null} persons studied are LW-negative (Beck, 1973) supports this view, as does the fact that a person can

FIG. 3. Probable biosynthetic pathway for Rhesus and LW. (Adapted from Giblett, 1969.)

have Rhesus antigens and yet be LW-negative (Levine *et al*, 1963).

Other blood group systems

Nothing is known about the chemical structure of antigens or other known blood group systems or about their biosynthesis.

HEMPAS

In HEMPAS (congenital dyserythropoietic anaemia—type II) the red cell membrane is abnormal (Crookston, Crookston, and Rosse, 1972; Verwilghen *et al*, 1973). The term HEMPAS is an abbreviation of the descriptive title 'Hereditary Erythroblastic Multinuclearity with a Positive Acidified-Serum Test'. The bone marrow is hypercellular, with a predominance (up to 90%) of erythroblasts. Intermediate and late normoblasts may have from two to seven nuclei. Electron-microscopy of erythroblasts shows an extra linear structure parallel to the inside of the cell membrane.

HEMPAS red cells are agglutinated and lysed by an IgM antibody, anti-HEMPAS, present in many (acidified) sera, but not that of the patient. The red cells are strongly agglutinated by anti-i (this property is common to all forms of congenital anaemia) and lysed by both anti-I and anti-i. It is not yet clear whether the HEMPAS receptor is an abnormal membrane structure produced by the *HEMPAS* gene or whether it is a normal component exposed only in HEMPAS cells. Also unclear at present is the relationship, if any, between i and HEMPAS.

Leucocyte antigens

ABH antigens occur in both red and white blood cells (Race and Sanger, 1968), and I and i can easily be detected in lymphocytes (Shumak *et al.*, 1971).

The HL-A system is a complex immunogenic system controlled by genes at two or more loci. The ABH and HL-A systems are of major importance in human transplantation because their antigens are not confined to blood cells but are present in most tissue cells.

The red cell antigens Bg^a, Bg^b and Bg^c are probably identical with, or very similar to, the HL-A antigens 7, W17 and W28, respectively (Morton, Pickles, and Sutton, 1969; Morton *et al.*, 1971). Recent work by Nordhagen and Ørjasæter (1974), who used an AutoAnalyzer technique, have shown that other HL-A antigens may be present, probably in modified form, on red cells; apparently such antigens can be better demonstrated on reticulocytes than on mature cells.

Most clinical studies of HL-A antigens have been made on material obtained from cells by means of detergents, enzymic digestion or ultrasonic wave bombardment. These methods are harsh and may produce a heterogeneous population of proteins so that purification is difficult. Since the HL-A antigens present in serum are the same as those on cells, Billing and Terasaki (1974) have studied the HL-A antigens of serum and have found them to be glycoproteins with a molecular weight of 130 000. No evidence is available as to whether the active site on the molecule is carbohydrate or protein. Theoretical and experimental evidence (Reisfeld and Kahan, 1972), however, suggest that histocompatibility loci code for polypeptide chains rather than carbohydrate moieties. This would account for the number of variants which characterize the HL-A system and for the broad specificity of some HL-A antibodies.

Platelet antigens

ABH and HL-A antigens are present in platelets. There are also blood group systems exclusive to platelets: Ko, Pl^A, and Pl^B (Svejgaard, 1969). Nothing is known of their chemistry or biosynthesis.

Relationships of blood group systems to one another

Since the lipid, protein, and carbohydrate building stones of the red cell surface are limited in number, it is not really surprising that structural relationships between various blood group systems have been demonstrated.

The relationship between ABH and Lewis, which

has been mentioned above, is perhaps the easiest to understand because it involves the competitive action of independent genes on the same substrate. The relationship of I and i to ABH is not so clear. Indeed, Ii is an unsatisfactory blood group system because its genetics is obscure. Serological interaction between ABH and Ii has been known for some time because of the occurrence of antibodies with the specificity anti-HI, anti-A₁I, and anti-BI (Race and Sanger, 1968). The relationship has been ascribed to steric interaction (Chessin and McGinnis, 1968; Bird and Wingham, 1970b). As mentioned above, Feizi *et al.* (1971a) believe that the I-substance may be part of the ABH-Lewis precursor substance.

A relationship between H, Lewis, and I was demonstrated by the findings of an antibody with the specificity anti-ILe^bH (Tegoli *et al.*, 1971), and a relationship between P and I by the demonstration of anti-IP₁ (Issitt *et al.*, 1968). The discovery of the Luke blood group character (Tippett *et al.*, 1965) revealed an association between the ABH and P blood groups. A further indication of an ABH-P relationship is given by the reactions of the agglutinins from *Fomes fomentarius* or certain fish ova which were originally thought to be anti-B but which were later found also to be anti-P and therefore to react poorly or not at all with group B cells of the genotype *pp* (Anstee, 1972). All these reagents are specific for α -D-galactose which is the immunodominant sugar in B and P specificity. There is also a relationship between the Lutheran, Auberger, P₁, and i antigens (Crawford, Tippett, and Sanger, 1974).

A relationship between ABO and MN is suggested by the action of agglutinins from *Moluccella laevis* seeds (Bird and Wingham, 1970a). This agglutinin has the specificity anti-A+N. It is a single agglutinin reactive with both A, MM and O, NN cells, and which can be completely absorbed by either. No clear relationship between MN and I has yet been established; an MN-active glycoprotein was, however, reported by Dzierzkowa-Borodej, Lisowska, and Seyfriedowa (1970) to inhibit anti-I.

A curious association of ABH and Rh was manifested by the remarkable anti-D serum described by Ikin, Mourant, and Pugh (1953), which, when tested with red cells suspended in physiological saline solution, reacted only with D-positive cells of group A.

An MN-Rh relationship has been revealed by the fact that in the regulator type of Rh_{null} cells, the U, and to some extent s, antigens are impaired (Schmidt and Vos, 1967). Perhaps a common substrate is

FIG. 4. Diagrammatic representation of known genetic or structural relationships between various blood group systems, ———: established; - - - - - : not yet substantiated.

used by the genes of these two systems. En(a-) cells are sialic acid-deficient and therefore their M and N antigens are depressed. The apparently antithetical relationship between Rh and En(a-) is probably due to the enhanced agglutinability of En(a-) cells.

When it was discovered (Ruddle *et al*, 1972) that the Rhesus and Duffy gene loci were situated on the same chromosome (No. 1), many blood workers had to revise their fundamental concepts of the laws of inheritance. Since most family studies show Rhesus and Duffy to segregate independently with but occasional 'disturbance' (Mohr, 1953/1954), it is now quite obvious that genes carried on the same chromosome, but which are sufficiently far apart, appear to segregate independently. Genes that are close together on the same chromosome and therefore transmitted together are said to be *linked*, but those that are beyond measurable linkage distance are termed *syntenic*. The Rhesus and Duffy genes are *syntenic*.

The genetic association of Rhesus and Duffy explains a puzzling blood group mosaic reported by Jenkins and Marsh (1965). A healthy blood donor had two red cell populations: one R_1r , Fy(a+b+), and the other rr , Fy(a-b+). The rr , Fy(a-) component could not have arisen by the conventional mode of inheritance; some change must have occurred in chromosome No. 1. Re-investigation, how-

ever, revealed no visible chromosomal abnormality in peripheral lymphocytes (Marsh and Chaganti, 1973).

Another indication of an Rh-Duffy relationship is provided by the reactions of the antibody anti-Fy5 (Colledge, Pezzulich, and Marsh, 1973). This antibody reacts with cells which are either Fy(a+) or Fy(b+) but fails to react with Rh_{null} cells even when they are Fy(a+) or Fy(b+). These observations are not yet satisfactorily explained; it is possible that the Rh and Duffy genes act on the same substrate.

The relationship between Rh and LW has been mentioned in a previous section.

The A-Cad/Sd^a relationship is dependent on the fact that α -N-acetyl-D-galactosamine is the immunodominant sugar in both the A and Cad receptors.

The probable identity of red cell Bg and other groups with certain HL-A antigens has been mentioned above.

Some of these various relationship are entirely due to structural similarities, eg, A and Cad, or to structural defects, eg, impaired M and N in En(a-) cells. Other relationships, although primarily genetic, are also clearly structural in the sense that the genes compete for a common substrate, eg, ABH and Lewis.

Conclusion

Much knowledge has been obtained on the molecular organization of cell surface structures, many of which are of importance in genetics and in clinical medicine. Plant seed agglutinins (lectins) have made a very useful contribution to our understanding of cell surface topography.

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