Genetic Variants Involving the Major Membrane Sialoglycoprotein of Human Erythrocytes

STUDIES ON ERYTHROCYTES OF TYPE M^k, MILTENBERGER CLASS V AND M^s

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1. Membranes from erythrocytes heterozygous for the M^k and Miltenberger Class V (Mi.V) condition and membranes from erythrocytes homozygous for the M^s condition were studied by polyacrylamide-gel electrophoresis by using the periodate/Schiff stain, binding of radioiodinated lectins and labelling with lactoperoxidase. 2. Both the M^k and Mi.V conditions are associated with a decreased content of the major blood-group-MN-active sialoglycoprotein. 3. An unusual blood-group-M-active membrane component was found in Mi.V cells of appropriate genotype. No comparable component was found in M^k erythrocytes. 4. The M^s antigen appears to result from a modification of the MN-active sialoglycoprotein found in normal cells. Our results suggest that the M^s sialoglycoprotein contains fewer sialotetrasaccharides than does the normal sialoglycoprotein. This may result from changes in the amino acid sequence of the protein. 5. The results are discussed in relation to differences in the antigenic properties of M^k, Mi.V and M^s cells and their possible influence on the structure of the surface of each of these cells.

The study of erythrocyte membrane variants is one approach to investigating the function of the surface glycoproteins of the human erythrocyte. Several types of membrane variants could be expected, such as those in which a normal membrane glycoprotein is totally absent or present in lower amounts and those in which there is an alteration in the carbohydrate content or amino acid sequence of the glycoprotein. Erythrocytes of type En(a–) provide an example of the first type of variant (Tanner & Anstee, 1976b; Gahmberg *et al.*, 1976). These rare human erythrocytes completely lack the major membrane sialoglycoprotein (syn: glycophorin A; Tomita & Marchesi, 1975). Individuals homozygous for this inherited membrane defect do not have any known clinical abnormalities, suggesting that in these cases the presence of the major sialoglycoprotein is not essential. However, in En(a–) erythrocytes the other major surface glycoprotein has an altered carbohydrate composition, which may compensate for the lack of the major sialoglycoprotein (Tanner *et al.*, 1976). In contrast, human erythrocytes of type S–s– appear to contain a minor glycoprotein (PAS-3) which is substantially deficient in its carbohydrate content (Dahr *et al.*, 1977a; Tanner *et al.*, 1977).

The major erythrocyte sialoglycoprotein carries

Abbreviations used: SDS, sodium dodecyl sulphate; IgG, immunoglobulin G.

the blood-group-MN antigens and is the major contributor of cell-surface sialic acid. Other human erythrocytes having abnormal expression of blood-group-MN antigens and associated with lower amounts of cell-surface sialic acid are known (Race & Sanger, 1975). It was decided to study these unusual variants to determine the nature of the changes in these cells. Cells described as M^k (Metaxas & Metaxas-Bühler, 1964) are characterized by a hemizygous absence of MN and Ss antigens and lower sialic acid content. As yet, only individuals heterozygous for this condition have been described (Nordling *et al.*, 1969; Sturgeon *et al.*, 1970; Metaxas *et al.*, 1971; Luner *et al.*, 1975). Cells of the Miltenberger Classes have certain rare antigens associated with the MNSs blood-group system. Miltenberger Class V erythrocytes have a lower sialic acid content together with weak MN antigens, and again only the heterozygous condition is known. A rare antigen (Hil) is a characteristic of these cells (Crossland *et al.*, 1970; Metaxas *et al.*, 1972; Luner *et al.*, 1975). Cells of type M^s also have a lower sialic acid content (Nordling *et al.*, 1969; Luner *et al.*, 1975). M^s is a very rare allele of the MN-blood-group system and unlike M^k and Mi.V is defined by the occurrence of the specific antibody anti-M^s (Allen *et al.*, 1958). One homozygous M^sM^s individual is known (Metaxas-Bühler *et al.*, 1966).

In the present paper we show that the M^k and Mi.V conditions are associated with the loss of the major sialoglycoprotein in erythrocytes, whereas in M^s erythrocytes there is an alteration in the major sialoglycoprotein.

Materials and Methods

Whole blood of donors of genotype M^k/MS (donor M.W.), M^k/Ms (donor H.S.), M^k/Ns (donor A.H.), Ms^{Mi.V}/Ns (donor J.L.), MS/Ns^{Mi.V} (donor R.C.), M^s/Ns (donor J.G.), M^s/MS (donor G.G.) and M^s/M^s (donor K.G.) were provided by Dr. M. Metaxas, Swiss Red Cross, Zürich, Switzerland. A blood sample of type M^k/Ns (donor F.Q.; Sturgeon *et al.*, 1970) was provided by Dr. P. Sturgeon, Cedars-Sinai Medical Center, Los Angeles, CA, U.S.A. Dr. C. M. Giles, Blood Group Reference Laboratory, London, U.K., provided cells of type M^s/Ns^{Mi.V} (donor L.R.; Crossland *et al.*, 1970), Ns/Ns^{Mi.V} (donor H.S.), Miltenberger Class III cells (Mi.III) and anti-Hil serum. Dr. E. W. Ikin, Blood Group Reference Laboratory, London, U.K., provided rabbit anti-M, anti-M^s and anti-N blood-grouping sera.

The methods for the preparation of erythrocyte 'ghosts', radioiodination of erythrocytes with lactoperoxidase, neuraminidase treatment of erythrocytes and location of lectin-binding and anti-M-binding components have been described previously (Boxer *et al.*, 1974; Tanner & Anstee, 1976a; Anstee *et al.*, 1977). SDS/polyacrylamide-gel electrophoresis was done in two systems, those described by Fairbanks *et al.* (1971) and by Laemmli (1970).

Quantitative determination of the amounts of radioactive lectin bound after SDS/polyacrylamide-gel electrophoresis and the binding of ¹²⁵I after lactoperoxidase-catalysed labelling of intact erythrocytes and subsequent SDS/polyacrylamide-gel electrophoresis was done as described (Tanner *et al.*, 1977). The binding of lectins was measured in several separate experiments. In each case normal control 'ghosts' were run in parallel with those from variant cells. The experiments with *Maclura aurantiaca* (Osage orange) lectin used 100–200 µl of labelled lectin (containing 2 × 10⁶–5 × 10⁶ c.p.m. of ¹²⁵I/ml) with haemagglutination titres of 1:40–1:128 against normal human group O erythrocytes. The experiments with *Arachis hypogaea* (peanut) lectin used 50–75 µl of labelled lectin containing 5 × 10⁶–7 × 10⁶ c.p.m./ml with haemagglutination titres of 1:320–1:640 against neuraminidase-treated human group-O erythrocytes. Both control and variant erythrocytes were treated with neuraminidase and 'ghosts' prepared before SDS/polyacrylamide-gel electrophoresis and measurement of the binding of *Arachis hypogaea* lectin. The methods for densitometric scanning of polyacrylamide gels and measuring

radioactivity have also been described previously (Tanner *et al.*, 1977).

Effect of trypsin on the Hil antigen

Anti-Hil serum (20 µl) was incubated with 20 µl of a 2% suspension of erythrocytes for 2 h at room temperature (20–23°C) before being read for agglutination microscopically. Mi.III, Mi.V and En(a–) cells were recovered after storage in liquid nitrogen. Untreated MN cells, trypsin-treated MN cells and untreated En(a–) cells (G.W.) gave no agglutination under these conditions.

Effect of trypsin on the M^s antigen

Human anti-M^s was obtained by screening of random blood donors (340) against M^s/Ns indicator cells; six examples were found (frequency 1:57) with haemagglutination titres in the range 1→4. All the sera failed to react with trypsin-treated M^s/Ns erythrocytes in direct agglutination tests. For absorption tests 50 µl of anti-M^s serum was incubated with 20 µl of washed packed erythrocytes for 1 h at room temperature (20–23°C) for rabbit antiserum and at 4°C for human antisera. The mixture was then centrifuged at 900g for 5 min and the supernatants were examined by haemagglutination titration against M^s/Ns erythrocytes (recovered from liquid nitrogen for use).

Results and Discussion

Major sialoglycoprotein of M^k, Mi.V and M^s erythrocytes

Erythrocyte 'ghosts' from each of the cell types were examined by SDS/polyacrylamide-gel electrophoresis. No differences in the pattern of protein staining (Coomassie Blue) or the mobility of the protein bands were observed when compared with membranes from control cells. When the gels were stained for carbohydrate with the periodic acid/Schiff-base stain a substantial decrease in staining of the different forms of the major sialoglycoprotein (PAS-1 and PAS-2) was observed with the membranes from M^k and Mi.V erythrocytes, but only a slight decrease was observed for the M^sM^s erythrocytes (Table 1). In addition, the Mi.V erythrocytes appeared to have an increased content of glycoprotein PAS-2' (nomenclature described in Tanner & Anstee, 1976b). Although two different control normal erythrocytes contained 0.56 and 0.63 staining unit of glycoprotein PAS-2' per unit of protein (defined as described in Table 1), two separate Mi.V samples (H.S. and L.R.) contained 1.95 and 2.0 staining units of glycoprotein PAS-2' per unit of protein.

The PAS-1 band of M^sM^s cells showed a faster electrophoretic mobility than that of normal cells (Fig. 1). Since these are highly glycosylated proteins,

Table 1. Measurement of the amount of major sialoglycoprotein in erythrocyte 'ghosts' of types, *M^k*, *Mi.V* and *M^sM^s*
The content of the major sialoglycoprotein (PAS-1+PAS-2) of variant cells is expressed as a percentage of PAS-1+PAS-2 values obtained for normal controls. All the values obtained were normalized to the same amounts of membrane protein by measurement of the area of band 4.2 (Steck, 1974) on parallel gels stained for protein with Coomassie Blue and scanned at 550nm. Periodic acid/Schiff-base staining values were obtained by integration of the areas of the PAS-1 and PAS-2 peaks on gels stained for carbohydrate and scanned at 560nm. Data are expressed as the means $\pm$ s.d. for the numbers of determinations in parentheses. The amounts of radioactive *Maclura aurantiaca* lectin and *Arachis hypogaea* lectin bound to the PAS-1 and PAS-2 bands after SDS/polyacrylamide-gel electrophoresis were measured as described in the Materials and Methods section. The values were normalized to equivalent membrane protein concentrations as described above and the results are expressed as percentages of the binding found with normal control erythrocytes. The amount of ¹²⁵I incorporated into the PAS-1 and PAS-2 bands by lactoperoxidase-catalysed radioiodination of intact erythrocytes was measured after SDS/polyacrylamide-gel electrophoresis as described above. The values were normalized to equivalent membrane protein concentrations as described above and the results are expressed as percentages of the binding found with normal control erythrocytes.

Cell type		Periodic acid/ Schiff-base staining	Lectin binding		Lactoperoxidase labelling
			<i>Arachis hypogaea</i>	<i>Maclura aurantiaca</i>	
<i>M^k</i>	M.W.	55 $\pm$ 9 (6)	73	65	No data
	H.S.	55 $\pm$ 7 (5)	60	73	57
	A.H.	47 $\pm$ 6 (5)	65	40	51
<i>Mi.V</i>	J.L.	58 $\pm$ 2 (2)	66	53	17
	R.C.	53 $\pm$ 6 (4)	61	60	26
<i>M^sM^s</i>	K.G.	82 $\pm$ 2 (2)	85	{ 56 64 }	63

Fig. 1. SDS/polyacrylamide-gel electrophoresis of 'ghosts' from *M^s* and normal erythrocytes

Electrophoresis was in a slab containing 8 % acrylamide with an overlay of 5% acrylamide in the buffer system of Fairbanks *et al.* (1971). The gel was stained with the periodic acid/Schiff-base stain. (a) Donor A (normal); (b) *M^s/M* (G.J.); (c) donor B (normal); (d) *M^sM^s* (K.J.). A portion of a typical slab electrophoresis run is shown. Similar results were obtained in three separate slab runs.

mol.wt. of 68000, whereas the normal sialoglycoprotein showed an apparent mol.wt. of 72000. The PAS-1 band of membranes from a heterozygous *M^s* donor migrated as a broad band which could be partially resolved into two bands of approximately equal staining intensity with mobilities corresponding to those of the normal and *M^sM^s* sialoglycoproteins (Fig. 1).

The membranes of all these cell types were also examined after SDS/polyacrylamide-gel electrophoresis by using radioiodinated lectins (Tanner & Anstee, 1976a) and the extent of binding of the lectins to the major sialoglycoprotein is shown in Table 1. Both the *Arachis hypogaea* lectin and the *Maclura aurantiaca* lectin showed less binding to the bands corresponding to the major sialoglycoprotein in heterozygous cells of type *M^k* and *Mi.V*. With the *M^sM^s* homozygous cell, the binding of the *Arachis hypogaea* lectin to the major sialoglycoprotein was only slightly decreased compared with that obtained for the normal controls, but there was a greater decrease in the binding of the *Maclura aurantiaca* lectin.

The above techniques all detect sugar moieties of the major sialoglycoprotein. The periodic acid/Schiff stain predominantly detects sialic acid, whereas the *Arachis hypogaea* and *Maclura aurantiaca* lectins probably detect galactose and *N*-acetylgalactosamine respectively in the *O*-glycosidically linked oligosaccharides of the major sialoglycoprotein (Anstee *et al.*, 1977; Lotan *et al.*, 1975; Terao *et al.*, 1975; Dahr *et al.*, 1975).

The results in Table 1 are consistent with a decrease

molecular-weight estimates from SDS/polyacrylamide-gel electrophoresis are unreliable. However, on gels containing 8 % acrylamide, the *M^sM^s* homozygous sialoglycoprotein migrated with an apparent

in the amount of the major sialoglycoprotein in both M^k and Mi.V. cells to about half that found in normal erythrocytes. To determine whether or not the amount of polypeptide chain of the major sialoglycoprotein was altered, the intact variant erythrocytes were radioiodinated by using the lactoperoxidase technique, together with appropriate control cells. The amount of ¹²⁵I bound to the major sialoglycoprotein is shown in Table 1. Compared with normal erythrocytes, the incorporation of ¹²⁵I into the sialoglycoprotein of M^k cells was decreased by about the same proportion as the decrease in periodate staining and the binding of lectins.

The decrease in lactoperoxidase labelling of the major sialoglycoprotein observed in the intact M^k erythrocyte is quantitatively similar to that observed after SDS/polyacrylamide-gel electrophoresis by using the periodate-staining and lectin-binding methods. This is consistent with the interpretation that in heterozygous M^k cells the defect simply results in the absence of approximately half the normal amount of the major sialoglycoprotein. However, for Mi.V erythrocytes, which appear to have the same basic defect, there is a greater decrease in the lactoperoxidase labelling of the intact cells than would be expected from the periodate-staining and lectin-binding results if the surface change was simply the result of a decrease in the amount of the major sialoglycoprotein. The lactoperoxidase radioiodination reaction is dependent on the steric conditions operating at the surface of the erythrocyte. It may be that other changes, reflected in the unique serological properties of the Mi.V class (the presence of the Hil antigen and abnormal M antigen described below cause changes in the ability of the protein to be iodinated). Since we have no evidence to suggest

that the residual sialoglycoprotein in Mi.V cells differs from that of normal M^k cells, the altered susceptibility to radioiodination of the major sialoglycoprotein could be the indirect result of changes in the structure of other cell-surface components in the immediate vicinity of the major sialoglycoprotein.

In homozygous En(a-) cells (EnEn) there is a total absence of the blood-group-MN-active major sialoglycoprotein. However, cells from individuals heterozygous for this condition (En^aEn) show a decrease in the amount of the major sialoglycoprotein of the same order as that observed here for M^k and Mi.V heterozygotes (Dahr *et al.*, 1976; Anstee *et al.*, 1977). By analogy, our present results indicate that the inheritance of a deficiency of the major sialoglycoprotein is associated with the M^k and Mi.V situations. This is consistent with the absence of blood-group-MN antigens associated with the inheritance of the M^k condition (Metaxas & Metaxas-Bühler, 1964). However, weak blood-group MN antigens are associated with the inheritance of the Mi.V condition, so that cells of genotype M^{Mi.V}/N have weak blood-group M activity and those of genotype M/N^{Mi.V} have weak blood-group N activity (Metaxas *et al.*, 1972). This raises the question of whether this weak MN-antigen activity represents residual amounts of the major sialoglycoprotein or whether an alternative source of MN-antigen activity is present in these cells.

Weak MN activity of Mi.V cells

Absorption experiments (Table 2) were used to confirm the presence of weak M and weak N activity associated with the cells of genotype M^{Mi.V}/N and N^{Mi.V}/M respectively to ensure that the apparent

Table 2. *Blood-group MN status of Miltenberger Class V erythrocytes*

Anti-(blood-group M) serum (50 μ l) or anti-(blood-group N) serum (100 μ l) was incubated with 20 μ l of washed packed erythrocytes for 1 h at room temperature (20–23°C) for rabbit antisera and at 4°C for the human anti-M serum. The mixture was then centrifuged at 900g for 5 min and the supernatants were examined by haemagglutination against the appropriate cells. The grading +++, ++, +, (+), vw indicates the strength of agglutination observed in single tests with undiluted sera. Numbers in parentheses indicate the reciprocal of haemagglutination titration end points. The methods of trypsin treatment of intact erythrocytes and the source of indicator cells are given in the Materials and Methods section. The Mi.V cells were recovered from storage in liquid nitrogen before use.

Absorbing cell		Agglutination reaction of supernatant after absorption		
		Human anti-M/MM cells	Rabbit anti-M/MM cells	Rabbit anti-N/NN cells
M ^{Mi.V} /N	Untreated	vw	++ (2)	Nil
	Trypsin-treated	+++ (4)	+++	+++
M/N ^{Mi.V}	Untreated	Nil	Nil	(+) (1)
	Trypsin-treated	+++	+++	++
M/M	Untreated	Nil	Nil	++ (2)
	Trypsin-treated	+++	+++	++
N/N	Untreated	+++ (2)	+++ (8)	Nil
	Trypsin-treated	+++	+++	++

weak MN activities were not the result of non-specific reactions of the antisera. The weak M and weak N activity of these cells could be destroyed by prior treatment of the cells with trypsin. With the $M^{Mi.V}/N$ cells it was possible to determine directly the membrane component responsible for the weak M activity by incubation of the electrophoretically separated membrane components with rabbit anti-M serum. Fig. 2 shows that the material binding the anti-M antibody in these cells migrates as a broad band with a higher electrophoretic mobility than the dimeric form (PAS-1; Furthmayr & Marchesi, 1976) of the major sialoglycoprotein. Although the anti-M binding material overlaps the position corresponding to the monomeric form of the major sialoglycoprotein (PAS-2) and the weak MN activity is trypsin-sensitive (Table 2), the band is unlikely to be simply due to traces of monomeric form of the major sialoglycoprotein, since it is broader than that of the normal monomeric form. In addition, the antibody did not detect a component in the position expected for the dimeric form of the glycoprotein. The small peak with a mobility corresponding to the front of the PAS-1 band in the $Mi.V$ sample (Fig. 2) is also found in the control NN sample and probably represents non-specific binding. The periodic acid/Schiff base-stained gels of this sample show that the PAS-1 and PAS-2 forms of the N-active sialoglycoprotein were present in the ratio 0.8/1.0. Similar experiments with $M/N^{Mi.V}$ membranes (containing normal M sialoglycoprotein) gave PAS-1/PAS-2 ratios of 1.0.

The spectrum of binding of the anti-M antibody to the $Mi.V$ cells suggests that more than one component is responsible for the weak M antigen activity observed on the intact cells. The anti-M binding extends to a region with faster mobility than glycoprotein PAS-2, corresponding to the position of glycoprotein PAS-2'. This may be related to the altered amount of periodic acid/Schiff-base staining of glycoprotein PAS-2' in $Mi.V$ erythrocytes. However, it is clear that other components with a mobility slower than glycoprotein PAS-2' also carry M antigen activity in these $M^{Mi.V}/N$ erythrocytes.

It is clear that $M^{Mi.V}/N$ individuals retain the ability to synthesize the M antigen. However, these cells contain normal N-active sialoglycoprotein, and thus the latter cannot be a substrate for the product of the M gene in these individuals. This suggests that the M and N antigens are synthesized by independent processes. These results are not consistent with the suggestion that the N antigen is a substrate for the M gene (Springer & Huprikar, 1972). An entirely analogous situation is found in another sialoglycoprotein-deficient family [En(U.K.)], although in this case the weak M activity is found in another group of membrane components (Anstee *et al.*, 1977).

The blood-group M and N antigenic determinants

Fig. 2. Binding of rabbit anti-(blood-group M) to separated components of human erythrocyte 'ghosts' from normal and $M^{Mi.V}/N$ donors

The M-antigen-active components were detected by using the IgG fraction of rabbit anti-M followed by ^{125}I -labelled pig anti-(rabbit IgG) as described (Anstee *et al.*, 1977). SDS/polyacrylamide-gel electrophoresis was carried out on gels containing 8% acrylamide, 0.300 ml of the IgG fraction of rabbit anti-M (haemagglutination titre 1:16 against group O/MM erythrocytes) and 1.0 ml of ^{125}I -labelled pig anti-(rabbit IgG) [haemagglutination titre 1:128 when tested by the direct anti-globulin method against human erythrocytes coated with a rabbit anti-(human erythrocyte) serum] containing 2×10^6 c.p.m./ml were used. The scans show densitometer traces at 500 nm of radioautographs of dried slices of parallel gels. The regions corresponding to the PAS-1 and PAS-2 bands are indicated. ----, Control normal MM erythrocyte 'ghosts'; —, 'ghosts' from $M^{Mi.V}/N$ erythrocytes (donor J.L.); ····, control normal NN erythrocytes.

appear to have a complex nature which involves both defined carbohydrate structures and specific residues in the polypeptide sequence (Dahr *et al.*, 1977b; Wasniowska *et al.*, 1977; Lisowska, 1977; Furthmayr, 1978). In the light of this it is surprising that the two types of weak M activity observed in En(U.K.) and $M^{Mi.V}$ erythrocytes are present on different membrane components, since the presence of the antigens on these components implies that they must have structural similarities with each other and with normal M-active sialoglycoprotein.

It is interesting that blood-group N antigen activity

is known to be present on two independent erythrocyte membrane components in type NN cells (the major sialoglycoprotein and PAS-3; Dahr *et al.*, 1977b), implying the presence of structural similarities between the two components. The extent of the structural similarity has been established by Furthmayr (1978), who has shown that the *N*-terminal 23–25 amino acid residues are identical in the two glycoproteins and that this identity also extends to the sites of glycosylation. The identity of the two *N*-terminal sequences is striking, since at least eight residues of the adjoining sequence are totally different and the two proteins differ substantially at their *C*-termini (Marchesi *et al.*, 1976). The extent and total identity of this large sequence at the *N*-terminus of these proteins makes it unlikely that it is the result of evolutionary constraints operating only on this region of the proteins. The *N*-terminal regions could be derived from a recent duplication of a single gene together with the addition of different *C*-terminal regions to give rise to separate genes for the two glycoproteins. An alternative intriguing possibility is that the *N*-terminal regions of the two proteins arise from a common gene which is independent of the genes coding for the *C*-terminal region, perhaps by a mechanism analogous to the somatic translocation which appears to determine the sequence of antibody molecules (Gally & Edelman, 1970). There has been evidence that the genes for some other proteins are split at the DNA level (Breathnach *et al.*, 1977).

One can envisage two types of mechanism by which the different weak M activities observed in En(U.K.) and Mi.V cells might occur. They could result either from the synthesis and incorporation of new membrane components or from alterations in pre-existing structures in the membrane, resulting from the deficiency of the major sialoglycoprotein. One way by which this latter situation might occur is by changes in the glycosylation of pre-existing glycoproteins to give rise to these M antigens because of differences in the structure of the cell surface, which alter the accessibility of glycoprotein precursors.

If the M antigenic activity of these components reflects structural similarities as extensive as those found between the N-active major sialoglycoprotein and glycoprotein PAS-3, then they might be derived by the same mechanism. Since the weak M antigen activity occurs on four components of different molecular weight [three in the En(U.K.) family, and one in M^{Mi.V} cells] which are also different from the normal MM sialoglycoprotein, the possibility that the M-active regions of these proteins are derived from a common gene seems likely. This may result from genetic events which are a consequence of the sialoglycoprotein deficiency known to occur in these cells.

The Hil antigen

Miltenberger Class V cells are defined by the presence of the unusual antigen Hil, a characteristic shared by Miltenberger Class Mi.III and Mi.VI cells (Race & Sanger, 1975; Giles *et al.*, 1977). The presence of the Hil antigen is not associated with the lack of the major sialoglycoprotein found in Mi.V cells, since experiments similar to those described in Table 1 showed that the PAS-1+PAS-2 content (estimated by periodic acid/Schiff-base staining) of 'ghosts' from Mi.III erythrocytes was not significantly different from that of normal erythrocyte membranes. In addition the Hil antigen of intact Mi.III or Mi.V cells could not be inactivated by prior treatment of the cells with trypsin as assessed by direct agglutination tests. Luner *et al.* (1975) have described a slight increase in sialic acid content in cells of the Mi.III class.

Nature of the M^a antigen

Genetic evidence has indicated that the M^a antigen is a simple allele in the MN blood-group system, suggesting that the M^a antigen is present on the major sialoglycoprotein in these cells. Incubation of the electrophoretically separated components of M^a cells with rabbit anti-M^a serum showed that the M^a-active component had a similar mobility to the PAS-1 and PAS-2 forms of the major sialoglycoprotein in these cells (Fig. 3). Absorption experiments with

Fig. 3. Binding of rabbit anti-M^a to separated components of human erythrocyte 'ghosts' from normal and M^a/N donors

The method is as described in the legend to Fig. 1, except that 100 μ l of the IgG fraction of rabbit anti-M^a (haemagglutination titre 1:4 against group O/M^a/N erythrocytes) and 200 μ l of ¹²⁵I-labelled pig anti-(rabbit IgG) [haemagglutination titre 1:128 when tested by the direct anti-globulin method against human erythrocytes coated with a rabbit anti-(human erythrocyte) serum] containing 24×10^6 c.p.m./ml were used. The regions corresponding to the PAS-1 and PAS-2 bands are indicated.

both human and rabbit anti-M^s sera showed that the M^s antigen was susceptible to trypsin treatment of the intact cells. This is also consistent with the antigen being located on the major sialoglycoprotein. It has been reported that the total sialic acid content of M^sM^s cells is slightly decreased (by 4–12%), compared with normal cells (Luner *et al.*, 1975; Nordling *et al.*, 1969). The periodic acid/Schiff-base staining of the major sialoglycoprotein in M^sM^s homozygous samples (Table 1) is consistent with a decrease in the sialic acid content of the major sialoglycoprotein of the same order (approx. 10%), but it is clear that the decrease in periodate-staining content of these cells homozygous for the defect is much less than is observed in the heterozygous M^k and Mi.V cells. Similar results were obtained for the binding of the *Arachis hypogaea* lectin, which is thought to bind to the subterminal galactose residues of the alkali-labile sialotetrasaccharide of the major sialoglycoprotein (Lotan *et al.*, 1975; Terao *et al.*, 1975), the major sialic acid-containing moieties in these cells. Both these results suggest that although there may be some decrease in the content of alkali-labile oligosaccharides in the homozygous M^sM^s cells, there is no reason to suppose that there is any substantial decrease in the amount of the major sialoglycoprotein in these cells. Since these M^sM^s cells are homozygous for the defect, it is clear that the presence of this gene does not result in the loss of the sialoglycoprotein in a way comparable with that found in M^k and Mi.V cells. However, the major sialoglycoprotein of M^sM^s erythrocytes bound less *Maclura aurantiaca* lectin than did the normal sialoglycoprotein.

The structure of the M^sM^s glycoprotein itself is clearly different from that of the normal sialoglycoprotein in that it no longer carries MN antigen activity but carries the M^s antigen. The sialoglycoprotein molecule is thought to contain 15 sialotetrasaccharides. It is not known whether the *Maclura aurantiaca* lectin binds equally to all these tetrasaccharides or whether the tertiary structure causes preferential binding to particular oligosaccharides. However, our results suggest that the M^sM^s sialoglycoprotein is not grossly changed in its content of sialotetrasaccharides as estimated by periodate staining and the binding of the *Arachis hypogaea* lectin. The decreased binding of the *Maclura aurantiaca* lectin suggests that this lectin may preferentially bind to particular oligosaccharides in the normal MN-active sialoglycoprotein, one or more of which may be lost or altered in the M^sM^s glycoprotein. The altered electrophoretic mobility of the M^sM^s sialoglycoprotein would be consistent with a small change in the number of sialotetrasaccharides in the protein. These changes may reflect an alteration in the overall structure of the M^sM^s protein which could also explain the decreased ability of the protein

to be labelled with lactoperoxidase in the intact cell. The cause of this decreased labelling would thus be quite different from that observed in the sialoglycoprotein-deficient Mi.V erythrocytes.

The altered binding of *Maclura aurantiaca* lectin may also reflect the molecular changes which give rise to the M^s antigen and result in the loss of the MN antigens. It has been established that one or more sialotetrasaccharides are part of the MN antigen structure (Lisowska, 1977), and it may be that the *Maclura aurantiaca* lectin is, in fact, detecting the particular tetrasaccharide which is part of the MN determinant. An analogous situation appears to exist for the *Vicia graminea* lectin, which has a clear specificity for the blood-group N antigen and probably detects differences in the accessibility of a galactose in the particular sialotetrasaccharide of the MN determinant (Lisowska, 1977).

The molecular nature of the M^s determinant is unknown, although sialic acid is not essential (Springer & Stalder, 1961). However, since amino acid-sequence changes are associated with the difference between the M and N antigen determinants (Wasniowska *et al.*, 1977; Dahr *et al.*, 1977b; Furthmayr, 1978), it may be that the M^s antigen, being a simple allele within the MN-blood-group system, also involves changes in the amino acid sequence of the protein. The presence of two distinct bands in the M^s heterozygote (Fig. 1) would be consistent with the independent synthesis of the normal and M^s components. The decrease in sugar content of the M^s sialoglycoprotein could be the result of amino acid differences between the M^s and MN polypeptides.

The results presented here are consistent with a decrease in the major sialoglycoprotein of M^k and Mi.V erythrocytes of the same order as that observed for heterozygous En(a-) cells (En^sEn). However, the M^k condition is also associated with an absence of Ss antigens. Cells totally lacking Ss antigens are known (Race & Sanger, 1975), and previous studies have suggested that a minor sialoglycoprotein (PAS-3) has an abnormal carbohydrate content in these cells which is reflected in a substantially lower amount of periodate staining (Tanner *et al.*, 1977). If a similar defect is the cause of the lack of Ss antigens in M^k cells then one might expect a lesser ability of the PAS-3 band to take up the periodate stain. Dahr *et al.* (1977c) have reported that the periodate staining of bands PAS-1, PAS-2 and PAS-3 is decreased in M^k erythrocytes. Although their results on the amounts of PAS-1 and PAS-2 in M^k erythrocytes are in agreement with ours we have not been able to demonstrate consistently a decrease in the extent of periodate staining associated with glycoprotein PAS-3 in M^k samples. The M^k condition has been found only in Caucasian and Chinese populations (Race & Sanger, 1975), whereas the S-s- defect

Table 3. *Sialoglycoprotein deficiencies*

Inherited condition	Major MN-active sialoglycoprotein	Associated cell surface changes		
En(Finland)	Absent		En ^a antigen absent	Wr ^a and Wr ^b antigens absent
En(U.K.)	Absent	Abnormal M antigen	En ^a antigen absent	Wr ^a and Wr ^b antigens absent†
Mi.V	Absent	Abnormal M or N antigen Hil antigen present	En ^a status not known‡	Wr ^a and Wr ^b antigens possibly absent*
M ^k	Absent	Ss antigens absent	En ^a antigens absent†	Wr ^a and Wr ^b antigens absent†

* Based on low Wr^b antigen dosage studies (M. Metaxas, personal communication).

† Based on the observation that members of the En(U.K.) family who inherit both the En(U.K.) and M^k conditions have the phenotype En(a—) and Wr (a—b—) (Metaxas & Metaxas-Bühler, 1976; Issitt *et al.*, 1976).

‡ Studies of En^a antigen dosage cannot be performed with available anti-En^a sera, since these do not distinguish between the En^aEn^a and En^aEn types (Darnborough *et al.*, 1969; Furuhielm *et al.*, 1969).

appears to be restricted to Negroid and Indian populations (Lowe & Moores, 1972; Moores, 1972). It is possible that the molecular nature of the S—s—defect differs in the two cell types. The availability of a cell homozygous for the M^k defect would be of great help in resolving this question.

The discovery and classification of M^k, Mi.V and En(a—) cells has been based on their unique serological properties. Although further examination has shown that these cells all have some abnormality associated with the MN-blood-group antigens, the nomenclature does not clearly reflect this. It is clear that all these conditions share a common major difference from normal cells, the absence of the major MN-active sialoglycoprotein. Table 3 summarizes the relationship between these cell types. The other changes associated with the absence of the major sialoglycoprotein from each cell type are also noted where experimental data are available.

The presence of the Hil antigen and the absence of Ss antigens appear to be quite independent of the sialoglycoprotein deficiency, since sialoglycoprotein-deficient cells exist, e.g. En(Finland), which have none of these associated changes, whereas Mi.III erythrocytes (with a normal sialoglycoprotein content) are characterized by the presence of the Hil antigen. However, so far as experimental data are available, it appears that the presence of the En^a and Wr^a/Wr^b antigens is associated with the presence of the major sialoglycoprotein (Issitt *et al.*, 1976), although the En^a antigen, at least, does not appear to be located on the major sialoglycoprotein (Tanner & Anstee, 1976b). The nature of the components which carry En^a and Wr^a/Wr^b activity and their relationship to sialoglycoprotein deficiency remain to be elucidated.

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