Individuals lacking the Gerbich blood-group antigen have alterations in the human erythrocyte membrane sialoglycoproteins β and γ

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1. Membranes from erythrocytes with a new Gerbich (Ge)-negative phenotype (Leach phenotype), as well as those from two other Ge-negative phenotypes, were examined. 2. Whereas cells of the Leach phenotype apparently lack three minor sialoglycoproteins (β , β_1 and γ), the membranes of Ge–Yus– and Ge–Yus+ erythrocytes apparently lack β - and γ -sialoglycoproteins but contain additional diffusely migrating components of apparent M_r 30 500–34 500 and 32 500–36 500 respectively. 3. Immunoprecipitation experiments showed that the abnormal components of both Ge–Yus– and Ge–Yus+ erythrocytes reacted with two monoclonal antibodies, BRIC 4 and BRIC 10. These antibodies have been shown to react with sialoglycoproteins β and β_1 in normal erythrocytes. 4. Cytoskeletal preparations from Ge–Yus– and Ge–Yus+ erythrocyte membranes contained the abnormal components. In contrast with cells of the Leach phenotype, which are elliptocytic, Ge–Yus– and Ge–Yus+ were of normal shape, despite their apparent lack of β - and γ -sialoglycoproteins. It seems likely that the abnormal components in these cells contribute to their normal shape. 5. Ovalocytic erythrocytes were shown to incorporate more radioactivity in the sialoglycoprotein- β_1 region than normal erythrocytes after labelling by the periodate/ NaB^3H_4 technique. 6. It is suggested that abnormal components in Ge–Yus– and Ge–Yus+ erythrocytes result from chromosomal misalignment with unequal crossing-over at meiosis between the genes giving rise to β -, β_1 - and γ -sialoglycoproteins.

The shape, integrity and flexibility of the human erythrocyte is believed to be controlled by a submembranous cytoskeleton network [see Gratzer (1981) and Cohen & Branton (1981) for reviews]. The integral membrane protein band 3 forms a major anchor for this network via the linker molecule ankyrin (Bennett & Stenbuck, 1979). Recent evidence suggests that there are additional sites for the attachment of the cytoskeleton to the membrane. In particular, the minor sialoglycoproteins β (synonyms glycoconnectin and glycophorin C), β_1 , ϵ and γ remain associated with cytoskeleton preparations (Mueller & Morri-

son, 1981; Anstee *et al.*, 1984). In the case of β it has been suggested that band 4.1 may be the linking protein.

Two individuals, (P. L. and K. W.) with elliptocytic red cells, who apparently lack sialoglycoproteins β , β_1 and γ , have recently been described (Anstee *et al.*, 1984). The red cells of P. L. and K. W. lack the Gerbich blood-group antigen (Ge), absence of which is extremely rare except in certain areas of Papua–New Guinea (Race & Sanger, 1975; Booth & McLoughlin, 1972). Consequently, erythrocyte membranes from other Ge-negative individuals were tested to determine whether changes similar to those found in P. L. and K. W. were also present. We have studied two other Gerbich-negative erythrocyte types (denoted here as ‘Ge–Yus–’ for the type originally described in the individual Mrs. Gerbich and ‘Ge–Yus+’ for the type originally described in

Abbreviations used: Ge, Gerbich antigen; NBTS/BRIC, National Blood Transfusion Service/Bristol Immunochemistry; anti-Ge etc. anti-(blood-group Ge) antibody etc.; IgG, immunoglobulin G; SDS, sodium dodecyl sulphate; PAS, periodic acid/Schiff's base.

the individual Mrs. Yus; Race & Sanger, 1975).

Ge-Yus- and Ge-Yus+ cells are recognized by their reactions with anti-Ge. Anti-Ge of the type found in the serum of Mrs. Gerbich reacts with Ge+ and with Ge-Yus+ cells, but not with Ge-Yus- cells, whereas anti-Ge of the type found in the serum of Mrs. Yus and in most Ge-Yus- people reacts with Ge+ cells but not with Ge-Yus- or Ge-Yus+ cells. Both types lack the minor sialoglycoproteins β and γ , but each contains different abnormal sialoglycoprotein molecules, both of which appear to be associated with the cytoskeleton. These erythrocytes have a normal shape, in contrast with the elliptocytic cells of P. L. and K. W.

Materials and methods

Erythrocytes

The unusual erythrocytes of P. L. and K. W. have been briefly reported previously (Anstee *et al.*, 1984). We suggest that the phenotype represented by these cells be denoted the 'Leach' phenotype after the original propositus, P. L. The blood groups of Mrs. P. L. are as follows: O M + N + S - s + P₂ C + c - D + E - e + Lu(a - b +) Le(a + b -) Fy(a + b +) Jk(a + b -) Xg(a +) Co(a + b +) Au(a +). The cells are also K - k + * Kp(a - b + * c -) Ku + * Js(a - b +) K: - 11, - 17 Ul(a -) KL + K: w12,13,14,18,w19,22 and react with the following antisera to high-frequency antigens: anti-H, -U, -PP, P^K, -Rh17, -Rh29, -LW^a, -LW^{ab}, -Lu3, -Fy3, -Jk3, -Di^b, -Yt^a, -Co3, -Sc1, -In^b, -At^a, -Ch, -Rg, -Cr^a, -Tc^a, -Dr^a, -Cs^a, -Yk^a, -En^a, -Er^a, -Gy, -Hy, -I, -i, -JMH-Jr^a, -Kn^a, -McC^a, -Lan, -Ok^a, -Vel, -Wr^b, -Don, -Emm, -Gil, -Kur, -Pel, Inab, R. M. and V. G. serum. The blood groups of Mrs. K. W. are as follows: O M + N - S + s + P₁ C + c + D + E - e + Lu(a -) Le(a -) Fy(a + b +) Jk(a - b +) Xg(a +) Co(b -) Au(a +).

Erythrocytes from eight Ge-Yus- and four Ge-Yus+ unrelated individuals were used for serological studies. Those used for membrane analysis were: Ste. and Jac. (Ge-Yus-) siblings from Dr. A. Lundsgaard, Aalborg, Sygehus, Denmark; D. O. (Ge-Yus-) from Dr. Sondag-Thull, Centre de Transfusion de Liège, Liège, Belgium; Jai. (Ge-Yus-) from Dr. D. Chandanayingyong, Faculty of Medicine, Siriraj Hospital, Mahidal University, Bangkok, Thailand; Z.Y. (Ge-Yus+) from Dr. C. Levene, Reference Laboratory for Immunohaematology and Blood Groups, Ministry of Health, Jerusalem, Israel; Cho. (Ge-Yus+) from Dr. B. Cedergren, Umeå Hospital, Umeå, Sweden.

Blood from a donor with ovalocytosis (donor 1380) was kindly supplied by Dr. T. Talonu,

Papua-New Guinea Red Cross Blood Transfusion Service, Boroko, Papua-New Guinea. Tn erythrocytes (donor Ba) were kindly provided by Dr. D. Lee and Mr. C. Hodson, Blood Transfusion Centre, Lancaster, U.K.

Serological methods

Standard serological techniques were used. For indirect agglutination tests either anti-(human IgG) or polyspecific anti-(human globulin) reagents were used for human antibodies and anti-(mouse IgG) for monoclonal antibodies. Washed, packed cells (1 vol.) were trypsin-treated by using 0.25% crystalline trypsin (Sigma) solution (4 vol.) for 30 min at 37°C in 0.1 M-phosphate buffer, pH 8.0, papain-treated by using 0.1% papain (BDH activated papain solution) (4 vol.) (Löw, 1955) for 10 min at 37°C, Pronase-treated by using 0.25% Pronase (Boehringer) solution (4 vol.) for 30 min at 37°C, and neuraminidase-treated by using 1 vol. of neuraminidase (Wellcome Research Laboratories) in 3 vol. of 3 mM-NaHCO₃/0.15 M-NaCl, pH 6.5, containing 1 mM-CaCl₂ (3 vol.) for 60 min at 37°C.

Monoclonal antibodies

Monoclonal antibodies NBTS/BRIC 4 (IgG1) and NBTS/BRIC 10 (IgG1) were prepared by fusing the NS1 cell line with spleen cells from BALB/c mice immunized with intact erythrocytes. The antibodies were used as unprocessed ascitic fluid. BRIC 4 was obtained from a mouse immunized with erythrocytes from an homozygous Miltenberger Class V donor (F. M.). One intraperitoneal injection of 0.1 ml of 50% (v/v) red blood cells in 0.15 M-NaCl was given and repeated 10 months later, the fusion being carried out 3 days after the second immunization. BRIC 10 was obtained from a mouse immunized with A₁B erythrocytes. One intraperitoneal injection of 0.1 ml of 50% red blood cells in 0.15 M-NaCl was given and repeated 3.5 months later, the fusion being carried out 3 days after the second immunization. The methodology was as described by Parsons *et al.* (1982).

Biochemical methods

The methods of SDS/polyacrylamide-gel electrophoresis, periodate/NaB³H₄ labelling of intact erythrocytes, immunoprecipitation and cytoskeleton preparation were as described by Mueller & Morrison (1981), Ridgwell *et al.* (1983) and Anstee *et al.* (1984), except for the immunoprecipitations involving Ge-Yus- and Ge-Yus+ erythrocytes, where the antibody-coated ghosts were incubated with 9 vol. of 0.1 mM-EDTA, pH 8.0, at 37°C for 20 min and centrifuged

at 30000g for 40 min at 4°C before solubilization in detergent and addition of Protein A-Sepharose.

Results and discussion

P. L. and K. W.

The erythrocytes of P. L. and K. W. have been the subject of a brief previous report (Anstee *et al.*, 1984). Mrs. P. L. (Caucasian, age 52) was ascertained when blood was required for transfusion during cardiac surgery in January 1982. Although she had not previously been pregnant or transfused, she had antibody in her serum that reacted with all of the erythrocyte samples tested. This antibody was identified as anti-Ge by Dr. C. M. Giles, who also showed that the erythrocytes of Mrs. P. L. were Ge-Yus-. The blood groups of Mrs. P. L. are given in the Materials and methods section. Her cells gave remarkably weaker reactions than those of controls with anti-k, -Kp^b, -Ku, -K12 and -K19, and failed to react with anti-K11. Erythrocytes of the Ge-Yus- phenotype generally show weaker-than-normal reactions with antisera specific for antigens related to the Kell blood-group system (Daniels, 1982). In contrast, Ge-Yus+ erythrocytes do not show this phenomenon. P. L. erythrocytes failed to react with 26 different human sera containing anti-Ge; five of the sera contained antibody of the type found in the serum of Mrs. Gerbich and 21 of the type found in the serum of Mrs. Yus (Race & Sanger, 1975). The serum of P. L. failed to react with six Ge-Yus- and three Ge-Yus+ erythrocyte samples and is therefore similar to the antibody found in the serum of Mrs. Yus. It failed to react with papain- or trypsin-treated Ge+ cells. P. L. serum also contains anti-Kell (K1).

Mrs. K. W. (Caucasian, age 64), has oat-cell carcinoma. The blood groups of Mrs. K. W. are given in the Materials and methods section. Her cells gave weaker reactions than those of control cells with antisera to certain Kell-related antigens, in an analogous manner to those described in detail for Mrs. P. L. above. The serum of Mrs. K. W.

contained a Gerbich antibody similar in specificity to that of Mrs. Yus.

Specificity of monoclonal antibodies BRIC 4 and BRIC 10

The erythrocytes of P. L. and K. W. did not react with either of the two monoclonal antibodies BRIC 4 and BRIC 10. These monoclonal antibodies reacted with normal erythrocytes, but not normal erythrocytes pretreated with neuraminidase, trypsin, Pronase or papain. The two antibodies react differently by indirect agglutination with Tn erythrocytes (Table 1). Tn erythrocytes lack the β -galactosyltransferase necessary for the completion of the *O*-glycosidically linked sialotetrasaccharides present on the erythrocyte sialoglycoproteins (Cartron *et al.*, 1978). This results in the presence of only the *O*-glycosidically linked *N*-acetylgalactosamine residues with some substitution with sialic acid. Thus, whereas the BRIC 10 determinant appears to depend on the sialic acid that is normally linked to galactose residues, BRIC 4 reactivity depends on sialic acid residues in a different location. However, both antibodies react with portions of the sialoglycoproteins that are released by trypsin treatment of intact Ge+ erythrocytes. These results are consistent with immunoprecipitation data, which suggests that both antibodies react with sialoglycoproteins β and β_1 (Anstee *et al.*, 1984). Sialoglycoprotein β is known to be cleaved by trypsin treatment to release a glycopeptide containing residues 1-47 from the *N*-terminus of the molecule (Dahr *et al.*, 1982). This region therefore probably contains the determinants on β recognized by BRIC 4 and BRIC 10. Since the sialic acid residues involved in the recognition sites for BRIC 4 and BRIC 10 are unique to β and β_1 , it seems likely that the specificity of the antibodies is determined, in part, by the structure of the polypeptide backbone. An analogous situation occurs with some antibodies to the blood-group-M and -N determinants, which require sialic acid for their reactivity but which depend on the polypeptide sequence at the *N*-

Table 1. *Specificity of monoclonal antibodies BRIC 4 and BRIC 10*

Treatments: U, untreated; T, trypsin-treated; N, neuraminidase-treated. Tn erythrocytes were from donor Ba (Gunson *et al.*, 1970). Abbreviation used: n.t., not tested.

Monoclonal antibody	Agglutination method	Erythrocytes	Reaction (+) or non-reaction (-)									
			Ge+		Ge-Yus-		Ge-Yus+		P. L.		Tn	
		Treatment	U	T	U	T	U	T	U	T	U	N
BRIC 4	Direct		-	-	-	-	-	-	-	-	+	-
	Indirect		+	-	+	+	+	-	-	-	+	-
BRIC 10	Direct		+	-	-	+	-	-	-	-	-	n.t.
	Indirect		+	-	+	+	+	-	-	-	-	n.t.

terminus of sialoglycoprotein α for their specificity (Judd *et al.*, 1979). If the specificities of BRIC 4 and BRIC 10 are dependent on amino acid sequence, then it is likely that β and β_1 , in part at least, have related amino acid sequences and are similarly orientated in the red-cell membrane. A similar close relationship between the amino acid sequences of the *N*-terminal regions of sialoglycoproteins α and δ is well known (Furthmayr, 1978; Dahr *et al.*, 1980).

Reactions of BRIC 4 and BRIC 10 with Ge-negative erythrocytes

Although BRIC 4 and BRIC 10 failed to react with P. L. and K. W. cells, both antibodies reacted with eight erythrocyte samples of phenotype Ge-Yus- and three of phenotype Ge-Yus+. However, whereas BRIC 10 agglutinated Ge+ erythrocytes directly, its reaction with both types of Ge- cells could only be detected indirectly by use of a second antibody (Table 1). In this respect the serological behaviour of BRIC 10 is similar to that of the monoclonal antibody GERO described by Daniels *et al.* (1983). The two Ge- types differed from each other in their reactions with both monoclonal antibodies after the erythrocytes had been treated with trypsin. Trypsin treatment destroyed reactivity with Ge-Yus+ erythrocytes, as it does with Ge+ cells. In contrast, the reactivity of the antibodies with Ge-Yus- erythrocytes was

not destroyed by trypsin treatment of the cells (Table 1).

Membrane components of Ge-negative erythrocytes

The membrane proteins of Ge-Yus- and Ge-Yus+ erythrocytes were examined by SDS/polyacrylamide-gel electrophoresis. No significant differences from the normal pattern were observed when the proteins were stained with Coomassie Blue; however, when the sialoglycoproteins were examined after staining with PAS reagent, it was evident that bands corresponding to sialoglycoproteins β and γ were absent from both types of Ge- cells (Figs. 1*b-1d*). There was, however, an additional diffuse PAS-staining band, of mobility intermediate between that of β and γ , that was absent from trypsin-treated Ge-Yus+ cells, but remained in trypsin-treated Ge-Yus- cells (Figs. 1*f-1h*). Further examination of these erythrocytes was carried out by using the periodate/boro[^3H]hydride method for labelling the sialoglycoproteins. The results (Figs. 2*b*, 2*c*, 2*i* and 2*j*) confirmed the absence of β and γ from both cell types and further demonstrated the presence of a strongly labelled diffuse band of mobility intermediate between that of normal β and γ . This band had a different electrophoretic mobility in the two types of Ge-negative cells. In Ge-Yus- erythrocytes (donors D. O. and Jai.) the band was spread over the apparent M_r range of 30 500–34 500 (mean

Fig. 1. SDS/polyacrylamide-gel electrophoresis of membranes from normal and Gerbich-negative erythrocytes. Electrophoresis was in a slab containing 10% (w/v) acrylamide with an overlay of 3% acrylamide, the buffer system of Laemmli (1970) being used. Trypsin treatment of intact erythrocytes was performed as described by Tanner *et al.* (1980). The samples were all run on the same gel and 150 μg of protein was applied to each slot. The gel was stained with the PAS stain. a, Membranes from normal Ge+ erythrocytes; b, membranes from Ge-Yus- erythrocytes (donor Ste); c, membranes from Ge-Yus- erythrocytes (donor Jac); d, membranes from Ge-Yus+ erythrocytes (donor Z. Y.); e, membranes from erythrocytes of Leach phenotype (donor P. L.); f, membranes from trypsin-treated Ge-Yus- erythrocytes (donor Jac); g, membranes from trypsin-treated Ge-Yus- erythrocytes (donor Ste); h, membranes from trypsin-treated Ge-Yus+ erythrocytes (donor Z. Y.); i, membranes from trypsin-treated normal Ge+ erythrocytes; j, membranes from normal Ge+ erythrocytes.

Fig. 2. Autoradiographs of membrane components from periodate/ NaB_3H_4 -labelled intact erythrocytes

Samples a–g and h–n are from different experiments. Where given, the number of days given in parentheses indicates the time of exposure for the autoradiographs. Tracks g and n are more-intense exposures of tracks a and h respectively to emphasize the minor labelled bands. a–g: a, Solubilized erythrocyte membranes (5 days); b, solubilized Ge–Yus+ (donor Cho) erythrocyte membranes; c, solubilized Ge–Yus– (donor D. O.) erythrocyte membranes; d, components from Ge–Yus+ (donor Cho) erythrocytes precipitated by monoclonal antibody BRIC 4; e, components from Ge–Yus– (donor D. O.) erythrocytes precipitated by monoclonal antibody BRIC 4; f, components from normal erythrocytes precipitated by monoclonal antibody BRIC 4; g, solubilized normal erythrocyte membranes (14 days). h–n: h, Solubilized normal erythrocyte membranes (5 days); i, solubilized Ge–Yus+ (donor Cho) erythrocyte membranes; j, solubilized Ge–Yus– (donor D. O.) erythrocyte membranes; k, components from Ge–Yus+ (donor Cho) erythrocytes precipitated by monoclonal antibody BRIC 10; l, components from Ge–Yus– (donor D. O.) erythrocytes precipitated by monoclonal antibody BRIC 10; m, components from normal erythrocytes precipitated by monoclonal antibody BRIC 10; n, solubilized normal erythrocyte membranes (14 days).

of four determinations), whereas in Ge–Yus+ erythrocytes the band corresponded to an apparent M_r range of 32500–36500 (mean of two determinations). The diffuseness of these abnormal bands made it impossible to ascertain whether any changes in the β_1 and ϵ bands occurred in these cells. In the Ge–Yus– sample from donor D. O., a sharp labelled band was also present (marked with an asterisk in Figs. 2c and 2j). This band was not immunoprecipitated by the monoclonal antibodies BRIC 4 and BRIC 10 (Figs. 2e and 2l). The significance of this band is unclear. Since the cells were received in poor condition, it may be a proteolytic artefact. The membranes from Ge–Yus– cells (obtained in good condition) from donor Jai. (Fig. 4a) did not show this band, although a minor component of similar apparent M_r was apparent in cytoskeletons prepared from these cells (Fig. 4b). This could be clarified if further samples from donor D. O. become available.

BRIC 4- and BRIC 10-reactive components in Ge-negative erythrocytes

Immunoprecipitation of BRIC 4- and BRIC 10-reactive components from Ge– erythrocytes labelled by using the periodate/boro ^3H hydride method showed that the abnormal components of both types of Ge-negative erythrocytes were specifically precipitated by both monoclonal antibodies (Figs. 2d, 2e, 2k and 2l).

Since BRIC 4 and BRIC 10 react with abnormal components in both types of Ge– cells, these antibodies are clearly not directed at either the Gerbich antigen or the Yus antigen. P. L. cells reacted with a large number of antibodies to high-incidence-blood group antigens (see the Materials and methods section). As BRIC 4 and BRIC 10 do not react with P. L. erythrocytes they do not detect any of the common blood-group antigens found to be present on P. L. cells. The apparent lack of β , β_1 and γ in P. L. and K. W. cells suggests that the Ge

antigen is present on one or more of these components in normal cells. Although both Ge-Yus- and Ge-Yus+ erythrocytes lack β and γ , we are unable to ascertain whether or not they also lack β_1 (and/or ϵ), since the abnormal components present in these cells have an electrophoretic mobility which masks the β_1 region and so we cannot eliminate β_1 as a possible carrier of the Ge antigen. Since the only apparent differences between the membrane components of Ge-Yus- and Ge-Yus+ erythrocytes are the different apparent M_r values and trypsin-sensitivities of the abnormal components, we conclude that the Yus antigen is likely to be located on the abnormal sialoglycoprotein component of Ge-Yus+ cells.

Cytoskeletal components of Ge-negative erythrocytes

Cytoskeleton preparations were made from periodate/boro[^3H]hydride-labelled Ge- erythrocytes and examined by SDS/polyacrylamide-gel electrophoresis. These results showed that the cytoskeletons of both Ge- types were selectively enriched in the abnormal components [Figs. 4(a)-

(4d) show the results for Ge-Yus- membranes]. Examination of blood smears from both Ge-Yus- and Ge-Yus+ individuals by optical microscopy showed no evidence for abnormal-shaped erythrocytes. This is in contrast with the presence of elliptocytic cells in blood smears from P. L. and K. W. (Anstee *et al.*, 1984), and suggests the possibility that the abnormal components found in both Ge-Yus- and Ge-Yus+ cells compensate for the shape defect that would otherwise have occurred due to the lack of β , γ and possibly β_1 in these cells. It may be that the abnormal components can provide an alternative source of membrane attachment sites for the cytoskeleton in these cells.

Membrane components of erythrocytes from an individual with ovalocytosis

In view of the possible association of β , β_1 and γ with erythrocyte shape, these membrane components in other abnormally shaped cells were examined. Ovalocytosis is common amongst Melanesians (as is the Ge-negative phenotype), and the

Fig. 3. SDS/polyacrylamide-gel electrophoresis of ovalocyte membranes

Where appropriate the period of autoradiography is given in parentheses. Tracks e and f are more intense exposures of tracks c and d respectively to emphasize the minor labelled bands. a, PAS stain of solubilized ovalocyte erythrocyte membranes (conditions were as given in the legend to Fig. 1); b, PAS stain of solubilized normal erythrocyte membranes. c-f, Autoradiographs of membrane components from periodate/ NaB^3H_4 -labelled intact erythrocytes: c, solubilized ovalocyte membranes (1 day); d, solubilized normal erythrocyte membranes (1 day); e, solubilized ovalocyte membranes (5 days); f, solubilized normal erythrocyte membranes (5 days). g, Components from ovalocytes precipitated by monoclonal antibody BRIC 10; h, components from normal erythrocytes precipitated by monoclonal antibody BRIC 10; i, components from ovalocytes precipitated by monoclonal antibody BRIC 4; j, components from normal erythrocytes precipitated by monoclonal antibody BRIC 4.

ovalocytic erythrocytes of these Gerbich-positive individuals are extremely refractory to invasion by *Plasmodium falciparum* malaria (Booth *et al.*, 1977; Kidson *et al.*, 1981). SDS/polyacrylamide-gel electrophoresis of membranes from ovalocytes did not show any significant differences from normal erythrocytes when stained with either the PAS stain (Figs. 3a and 3b) or Coomassie Blue (results not shown). However, when ovalocytes were labelled by the periodate/boro[^3H]hydride method, no difference was observed in the major labelled components, but there was an increased intensity of labelling in the region of β_1 and ϵ (Fig. 3e). Immunoprecipitation with BRIC 4 and BRIC 10 showed that these antibodies precipitated similar amounts of β from both samples, but markedly more radioactivity in the region of β_1 from the ovalocytes (Figs. 3g and 3i). These results suggest that ovalocytes differ from normal erythrocytes in that they incorporate more radioactivity in the region of β_1 . Cytoskeletons prepared from ovalocytes also had increased labelled material in the

region of β_1 (Fig. 4h). This increased labelling may reflect an increase in the number of copies of this cytoskeletal component, which may be associated with the altered shape of ovalocytes. It is possible that an increased number of attachment sites for the cytoskeleton to the membrane may be the cause of the resistance of these cells to invasion by *P. falciparum* malaria.

The nature of the abnormal components in Ge-negative and ovalocytic erythrocytes

Since the abnormal components in Ge–Yus– and Ge–Yus+ cells share with β and β_1 the antigenic determinants recognized by monoclonal antibodies BRIC 4 and BRIC 10, it seems likely that these components are structurally related to β and β_1 . In both types of Ge– cells the presence of the abnormal components is associated with the absence of β and γ . This situation shows a striking analogy with that occurring in Miltenberger Class V erythrocytes, where an unusual hybrid sialoglycoprotein [$(\alpha\text{--}\delta)^{\text{MIV}}$] is found in association

Fig. 4. Cytoskeleton preparations from Gerbich-negative and ovalocyte periodate/ NaB^3H_4 -labelled intact erythrocytes. Autoradiographs a–d and e–l are from different experiments. The period of autoradiography is indicated in parentheses. Autoradiographs are shown for: a, solubilized Ge–Yus– (donor Jai) erythrocyte membranes (14 days); b, cytoskeletons prepared from Ge–Yus– (donor Jai) erythrocyte membranes (14 days); c, solubilized normal erythrocyte membranes (14 days); d, cytoskeletons prepared from normal erythrocyte membranes (14 days); e, solubilized ovalocyte membranes (3 days); f, cytoskeletons prepared from ovalocyte membranes (3 days); g, solubilized ovalocyte membranes (21 days); h, cytoskeletons prepared from ovalocyte membranes (21 days); i, solubilized normal erythrocyte membranes (3 days); j, cytoskeletons prepared from normal erythrocyte membranes (3 days); k, solubilized normal erythrocyte membranes (21 days); l, cytoskeletons prepared from normal erythrocyte membranes (21 days).

with the absence of sialoglycoproteins α and δ (Anstee *et al.*, 1979). The *N*-terminus of $(\alpha-\delta)^{\text{MiV}}$ is derived from the *N*-terminus of α , whereas the *C*-terminus originates from the *C*-terminus of normal δ . This is thought to arise from chromosomal misalignment by unequal crossing-over between the α and δ genes at meiosis, as is found in haemoglobin Lepore (Anstee *et al.*, 1982). In the case of Ge-Yus- and Ge-Yus+ erythrocytes, the abnormal components would be hybrid sialoglycoproteins resulting from cross-overs between the β and/or β_1 and/or γ genes. This would imply that these genes are adjacent on the same chromosome. Our results suggest the possibility that the Yus antigen (which has not been proved to be present on normal erythrocytes) originates from a new protein structure generated by the putative cross-over event, leading to an abnormal hybrid molecule. A similar unusual antigen (Hil) is found in association with the $(\alpha-\delta)^{\text{MiV}}$ hybrid sialoglycoprotein (Anstee *et al.*, 1979). Sialoglycoprotein β is known to be orientated in the membrane with its *N*-terminus extracellular. The trypsin-sensitivity of the BRIC 4 and BRIC 10 determinants on β and β_1 and on the abnormal component of Ge-Yus+ erythrocytes suggests that the *N*-terminus of the abnormal component may be derived from β or β_1 . The *C*-terminus of the hybrid molecule could be derived from γ . A similar explanation could account for the origin of the abnormal component of Ge-Yus- erythrocytes.

A consequence of the putative cross-over events giving rise to the Lepore-like Ge-negative hybrid sialoglycoproteins would be the generation of the complementary anti-Lepore-like genotype resulting in the retention of normal β , β_1 and γ genes and the additional presence of an anti-Lepore-type sialoglycoprotein. The increased labelling that we observed in the region of β_1 in ovalocytes could result from the presence of increased amounts of β_1 and/or an additional anti-Lepore hybrid sialoglycoprotein. The abnormal shape and increased thermal stability (Kidson *et al.*, 1981) of ovalocytes could result from the additional presence of sialoglycoproteins associated with the cytoskeleton. Anti-Lepore-type $\delta-\alpha$ hybrid sialoglycoproteins are known to occur (Anstee *et al.*, 1982). Clearly, further structural information on the relationship between β , β_1 and γ will be necessary to validate these hypotheses and eliminate other possibilities.

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