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Structural relationships between human erythrocyte sialoglycoproteins β and γ and abnormal sialoglycoproteins found in certain rare human erythrocyte variants lacking the Gerbich blood-group antigen(s)

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1. The human erythrocyte membrane sialoglycoproteins β and γ are important for the maintenance of the discoid shape of the normal erythrocyte. In this paper we show that the human erythrocyte sialoglycoproteins β and γ (hereafter called β and γ) are structurally related. Rabbit antisera produced against purified β and β_1 and rendered specific to the cytoplasmic portion of these proteins also react with the cytoplasmic portion of γ. Some human anti-Gerbich (Ge) sera react with the extracellular portion of both β and γ. This reactivity is shown to be directed towards a common epitope on β and γ. However, most anti-Ge sera do not react with β, but react with an extracellular epitope only present on γ. 2. All individuals who lack the Ge antigens lack β and γ. In some cases abnormal sialoglycoproteins are present in the erythrocytes, and these are shown to be structurally related to β and γ. Rabbit antisera raised against the purified abnormal sialoglycoprotein from a Ge-negative erythrocyte type reacted with the cytoplasmic portion of both β and γ. 3. Unlike normal β and γ, the abnormal sialoglycoproteins found in Ge-negative erythrocytes migrate as a diffuse band on SDS/polyacrylamide-gel electrophoresis. Studies using endoglycosidases suggest that the diffuse nature of these bands results from carbohydrate heterogeneity and that the abnormal sialoglycoproteins contain *N*-glycosidically linked oligosaccharides with repeating lactosamine units. Such polylactosamine chains are not present on normal β or γ.

INTRODUCTION

The human erythrocyte membrane contains four predominant sialoglycoproteins (denoted α, β, γ and δ; Anstee *et al.*, 1979). Although the most abundant of these (α and δ) have been extensively studied, their function is, as yet, unknown. In contrast, there is substantial evidence for the involvement of β and γ in maintaining the shape of the human erythrocyte. Both β and γ are associated with the skeleton of normal erythrocytes. Mueller & Morrison (1981) found that β was absent from skeletons prepared from the erythrocyte membranes of a patient with hereditary elliptocytosis with band-4.1 deficiency. They suggested that the cytoplasmic portion of β provides a membrane attachment site for the skeleton through band 4.1. This suggestion has been supported by the finding of five individuals from three apparently unrelated kindred with elliptocytosis whose erythrocyte membranes lack β, γ and a minor component denoted β_1 (Anstee *et al.*, 1984*a*; Daniels *et al.*, 1986). The erythrocytes of these latter individuals lack the very common Gerbich (Ge) antigen(s) and are described as 'Leach phenotype' (Anstee *et al.*, 1984*b*). Two other erythrocyte types which also lack the Ge antigen have been described (Ge type and Yus type), and membranes derived from these erythrocytes also apparently lack β and γ. However, the membranes of both these types possess abnormal sialoglycoprotein molecules and the erythrocytes have normal shape (Anstee *et al.*, 1984*b*). We have suggested that these abnormal sialoglycoprotein molecules are hybrid molecules with their *N*-terminus derived from β and their *C*-terminus derived from γ and that they function in place of β and γ in these cells.

In the experiments reported here we have used rabbit antisera which react with sialoglycoproteins β and γ to confirm the absence of β and γ from Ge-negative erythrocytes and to show that the cytoplasmic portion of β, γ and the abnormal components of Ge-type and Yus-type erythrocytes are structurally related; we also show that the abnormal components are more extensively *N*-glycosylated than is normal β. In addition, we demonstrate that the extramembranous portion of γ is immunologically distinct from that of β.

MATERIALS AND METHODS

Murine monoclonal anti-β (BRIC 10) antibody was as described by Anstee *et al.* (1984*a*,*b*). The twenty different human anti-Ge sera were gifts from a variety of sources, as were erythrocyte samples from Ge-negative donors of the Ge type and the Yus type. SDS/polyacrylamide-gel

Abbreviations used: β and γ, sialoglycoproteins β and γ; PBS, phosphate-buffered saline, pH 7.4 (Dulbecco A; Oxoid, Basingstoke, Hants., U.K.); PMSF, phenylmethanesulphonyl fluoride; DTT, dithiothreitol; LDAO, lauryl (dodecyl) dimethylamine oxide; Ge, Gerbich; Endo F, endoglycosidase F (peptidyl *N*-glycosidase F).

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electrophoresis of erythrocyte membranes was carried out on gels containing 10% acrylamide with a 3%-acrylamide overlay using the method of Laemmli (1970). Immunoblotting was as described by Mallinson *et al.* (1986), except that 5% (w/v) bovine milk powder was used as the blocking agent. When human antisera were used, their binding was detected by using peroxidase-conjugated rabbit and goat anti-human IgG (Dako Ltd., High Wycombe, Bucks., U.K., or Bio-Rad, Richmond, CA, U.S.A.). The binding of rabbit antisera was detected by using the biotin–streptavidin–peroxidase system supplied by Amersham International, Amersham, Bucks., U.K. The production and use of endo-β-galactosidase (*Flavobacterium keratolyticus*) and Endo F preparations (*Flavobacterium meningosepticum*; Elder & Alexander, 1982) has been described in detail elsewhere (Tanner *et al.*, 1987). Immunoprecipitation was performed using ghosts prepared from erythrocytes labelled by the periodate/NaB^3H_4 method. Briefly, 1 vol. of intact erythrocytes was labelled with periodate/NaB^3H_4 (Gahmberg & Andersson, 1977) and ghosts prepared. Antibody preparations were incubated with labelled ghosts for 60 min on ice. The ghosts were then washed with 7 mM-sodium phosphate buffer, pH 7.4, before addition of 6 vol. of 1% Triton X-100/PBS (pH 7.4)/1 mM-PMSF at room temperature. After 15 min incubation, Sepharose–Protein A (Pharmacia) was added and the tubes were placed on a rotator at room temperature for 30 min. The Protein A–Sepharose beads were washed four times with 1% Triton/PBS (pH 7.4)/1 mM-PMSF before addition of sample buffer [20 mM-Tris (pH 8.0)/5 mM-EDTA/5% SDS/10% glycerol/Bromophenol Blue (0.1 mg/ml)/Pyronin Y (0.1 mg/ml)/5% 2-mercaptoethanol/2 mM-PMSF]). These immunoprecipitated preparations were incubated at 37 °C for 15 min and then in a boiling-water bath before SDS/polyacrylamide-gel electrophoresis.

Purification of β and the abnormal component from Ge-negative erythrocytes of the Gerbich type

Normal β was purified from erythrocytes available at the South Western Regional Blood Transfusion Centre, Bristol, U.K. The abnormal component from a Ge-negative donor of the Ge type (donor Guy) was purified from erythrocytes obtained from a donor at the Blood Transfusion Service, Boroko, Papua New Guinea. Ghosts were prepared from washed (0.15 M-NaCl) erythrocytes from a whole donation of blood ($\simeq$ 200 ml of packed erythrocytes) by the method of Dodge *et al.* (1963). The ghosts were then extracted with 5 or 6 vol. of 0.1% Triton X-100/1 mM-DTT/1 mM-EDTA/1 mM-PMSF at pH 7.4 on ice for 15 min (Owens *et al.*, 1980). The pellet obtained after centrifugation at 30000 ***g*** for 30 min at 4 °C was washed twice in 7 mM-sodium phosphate buffer/1 mM-DTT/1 mM-EDTA/1 mM-PMSF, pH 7.4, and the sialoglycoproteins extracted as described by Anstee & Tanner (1974). Briefly, the pellet was suspended in ice-cold 20 mM-sodium phosphate buffer/1 mM-PMSF pH 7.0 (50 ml), mixed and incubated on ice for 5 min. An equal volume of ice-cold n-butanol was added, the whole vigorously shaken and then incubated on ice for 15 min. After centrifugation at 25000 ***g*** for 10 min at 4 °C, the aqueous phase was recovered, dialysed exhaustively against distilled water and freeze-dried. Aliquots of the freeze-dried material were dissolved in 50 mM-Tris/HCl containing 150 mM-NaCl, 0.5% LDAO (Fluka, Germany) and 1 mM-PMSF, pH 7.0, and applied to an affinity column comprising murine monoclonal anti-β antibody bound to Sepharose equilibrated in the same buffer. Elution of bound material was achieved with 50 mM-triethylamine/0.5% LDAO/1 mM-PMSF, pH 11.0. The eluted material was immediately collected into 1 M-Tris/HCl, pH 7.4, dialysed extensively against distilled water and freeze-dried. The material not retained by the column was used as a γ-rich preparation in haemagglutination-inhibition assays.

Preparation of rabbit antisera to β and the abnormal component from Ge-negative erythrocytes of the Gerbich type

Antibodies were produced in rabbits by the protocol described previously (Mawby *et al.*, 1981). The rabbits were bled 10 days after the third and subsequent subcutaneous injections, the serum separated and heated to 56 °C for 30 min. To remove those antibodies specific for the extracellular portion of β and/or γ, the rabbit sera were absorbed with normal intact erythrocytes until no haemagglutinin (direct or indirect) activity remained. This serum was dialysed against PBS, pH 7.4, and the IgG fraction was purified on a Protein A–Sepharose column (Langone, 1982). Absence of haemagglutinating activity against intact human erythrocytes was confirmed on the final preparations.

RESULTS AND DISCUSSION

β, β_1, γ and the abnormal sialoglycoprotein components of Gerbich (Ge) negative erythrocytes are structurally related

Rabbit antisera were produced to purified normal β and β_1 (serum E) and to the purified abnormal component of Ge-negative erythrocytes of the Ge type (serum G) as described in the Materials and methods section. Each rabbit serum was absorbed with intact human erythrocytes to remove antibody activity to the extracellular surface and the post-absorption serum used in immunoprecipitation experiments with erythrocyte ghosts prepared from periodate/NaB^3H_4-labelled erythrocytes. Serum E immunoprecipitated β from normal ghosts, but not from ghosts of Leach phenotype (Figs. 1*a* and 1*b*). Inspection of the fluorograph demonstrated that β_1 and γ were also immunoprecipitated from normal ghosts. Serum G immunoprecipitated β, β_1 and γ from normal ghosts, but not from Leach-phenotype ghosts. Only the abnormal component was immunoprecipitated from Ge-negative ghosts of the Ge type (Figs. 1*c*, 1*d* and 1*e*). Immunoblotting with serum E and serum G demonstrated that both sera reacted with β and γ in normal ghosts and with the abnormal component of Ge-negative erythrocyte membranes of the Ge and Yus types. No bands corresponding to β or γ were found in any of the three Ge-negative cell types (Leach type, Ge type and Yus type; results not shown). The results obtained with serum G suggest that the cytoplasmic portion of the abnormal component of Ge-negative erythrocytes of the Ge type shares structural homology with normal β, β_1 and γ and with the abnormal component of Ge-negative erythrocytes of the Yus type.

The results obtained with sera E and G are consistent with previous evidence that β and γ are absent from

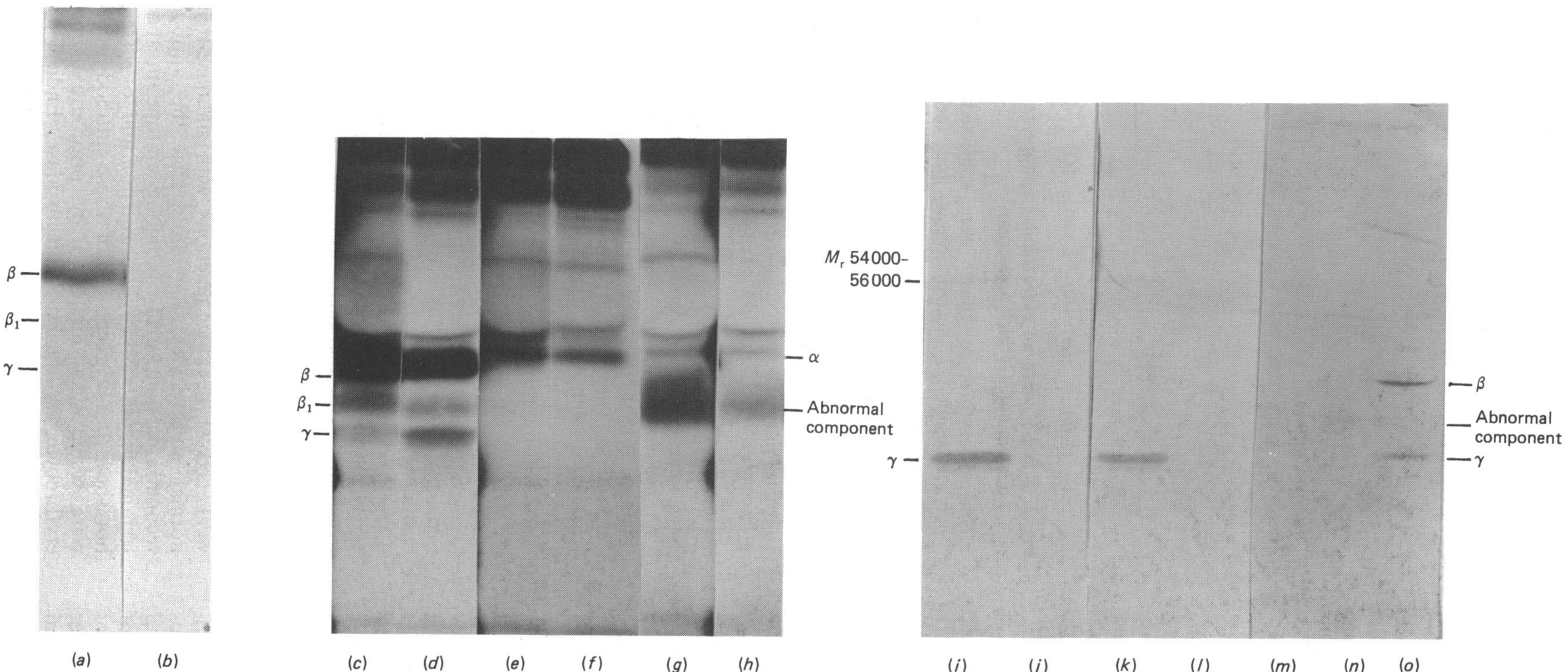

Fig. 1. Reactivity of rabbit and human antisera with normal and Ge-negative erythrocytes

(*a*)–(*h*) Fluorographs of immunoprecipitates from erythrocytes labelled by using the periodate/NaB^3H_4 method. Tracks *a* and *b*, immunoprecipitation of normal (*a*) and Leach-phenotype (*b*) erythrocytes with rabbit E serum (pre-absorbed with intact erythrocytes). Although not evident in the Figure, inspection of the fluorograph showed the presence of weak bands corresponding to β_1 and γ in track (*a*) and not in track (*b*). Fluorography was for 1 month. Tracks (*c*)–(*h*), immunoprecipitation of normal, Leach-phenotype (donor P.L.) and Ge-negative erythrocytes of the Ge type (donor Jai from Dr. D. Chandanayingyong, Bangkok, Thailand) with rabbit G serum. Tracks (*c*) and (*d*), normal erythrocytes with unabsorbed (*c*) and absorbed (*d*) rabbit G serum. Tracks (*e*) and (*f*), Leach-phenotype erythrocytes with unabsorbed (*e*) and absorbed (*f*) rabbit G serum. Tracks (*g*) and (*h*), Ge-negative erythrocytes with unabsorbed (*g*) and absorbed (*h*) rabbit G serum. Prolonged fluorography (7 months) revealed several other bands of M_r greater than that of β in all three samples. Tracks (*i*)–(*o*), immunoblots using human anti-Ge antisera; (*i*) and (*j*), anti-Ge (WHA) with normal erythrocytes (*i*) and Leach-phenotype erythrocytes (*j*); (*k*) and (*l*), anti-Ge (SMI) with normal erythrocytes (*k*) and Leach-phenotype erythrocytes (*l*); (*m*)–(*o*) auto-anti-Ge (Rod) with Ge-negative erythrocytes of the Ge type [(*m*) donor M.G. from M. Mohammed, American Red Cross, San Jose, CA, U.S.A.], Ge-negative erythrocytes of the Yus type [(*n*) donor Cho from Dr. B. Cedergren, Uppsala, Sweden] and normal erythrocytes (*o*). Anti-Ge sera SMI and Rod were from V. Vengelen-Tyler, American Red Cross, Los Angeles, CA, U.S.A. Anti-Ge serum WHA was from S. Cornwall, Canadian Red Cross, Toronto, Canada.

Ge-negative erythrocytes of each of the three types. Membranes from erythrocytes of Leach phenotype also lack β_1. However, since the abnormal components of Ge and Yus type cells migrate in the region of β_1, it is not possible to determine if this component is absent from these cells.

Antibodies directed against Ge-related antigens may be found in the serum of Ge-negative individuals as naturally occurring antibodies or as antibodies produced in response to pregnancy or transfusion. Anti-Ge antibodies in individuals with normal Ge antigens (auto-anti-Ge antibodies) have also been described (for a review, see Reid, 1986). The specificity of the human anti-Ge antibodies was analysed by immunoblotting. In all, 19 different human serum samples containing anti-Ge and one containing auto-anti-Ge were studied. All these antisera reacted with sialoglycoprotein γ (see, e.g., Fig. 1). A reactive band of M_r 54000–56000 was also observed on the immunoblots, which may correspond to dimers of γ (Fig. 1). Four of the antisera, including the auto-antibody, also reacted with β, but none reacted with β alone. Three of these antisera (which reacted with both β and γ) also reacted with the abnormal component of Yus-type Ge-negative erythrocytes. The auto-antibody was one of these. The other antiserum (R.W., from Ms. V. Vengelen-Tyler, American Red Cross, Los Angeles, CA, U.S.A.), which reacted with both β and γ, was unusual in that it was obtained from serum of an individual whose erythrocytes apparently contained the abnormal Yus-type component. This abnormal component in the erythrocytes of this individual may, therefore, be different from the usual component found in most Yus-type erythrocytes. None of those antisera which reacted with γ alone also reacted with the abnormal sialoglycoprotein of Yus-type erythrocyte membranes. In haemagglutination-inhibition assays, the sera which reacted with γ alone using immunoblotting were also shown to be inhibited by a γ-rich sialoglycoprotein preparation, but not by purified β (results not shown). Since these sera react specifically with the extracellular portion of γ, this region of γ contains an epitope not present on β.

The experiment shown in Fig. 2 was carried out to determine if those antisera which recognize both β and γ react with an epitope common to the extracellular portion of the two sialoglycoproteins. Normal erythrocyte ghosts were subjected to SDS/polyacrylamide-gel electrophoresis, the separated components electrophoretically transferred to nitrocellulose and incubated with human anti-Ge serum (44017). The antibody molecules bound to the regions corresponding to β and γ were separately eluted and their reactivity towards β and γ determined by immunoblotting normal erythrocyte ghosts. The eluate recovered from the β region reacted with both β and γ (Fig. 2*d*). The eluate recovered from the γ region also reacted with both β and γ (Fig. 2*f*). Neither of the eluted antibodies reacted with immunoblots of Leach-phenotype ghosts (which lack both β and γ; Figs. 2*c* and 2*e*). As a control, a portion of nitrocellulose from a region containing components of M_r 15000–25000 was subjected to the same procedure; the eluted material showed no reactivity with normal erythrocyte ghosts (Fig. 2*h*). This result demonstrates that the extracellular portions of β and γ share a common epitope.

All the human anti-Ge sera failed to react with trypsin-treated normal erythrocytes and gave either weakened or negative reactions with sialidase-treated normal erythrocytes, suggesting that the antibodies recognize a sialic acid-containing epitope in the *N*-terminal region of sialoglycoprotein β and/or γ (results not shown). The reactivity of those antibodies which were tested was unaffected by prior treatment of erythrocytes with endo-β-galactosidase, Endo F or a combination of the two enzymes when immunoblots of the treated membranes were analysed (results not shown). These results are consistent with the absence of *N*-glycosidically linked sugar from γ and show that the

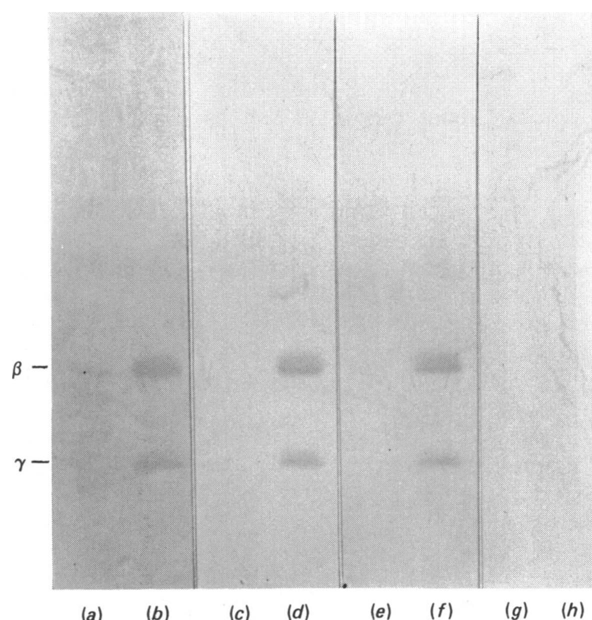

Fig. 2. β and γ share an extracellular epitope recognized by a human anti-Gerbich antiserum

Normal erythrocyte ghosts were subjected to SDS/polyacrylamide-gel electrophoresis and the separated components electrophoretically transferred to nitrocellulose and blocked with 5% bovine milk powder. The anti-Ge component of human serum 44107 (from J. Decker, American Red Cross Blood Services, Tucson, AZ, U.S.A.) was purified by absorption and elution from erythrocytes (Tanner *et al.*, 1987) and incubated with the nitrocellulose. The nitrocellulose was washed with 0.3% Tween/PBS, pH 7.4, and the regions corresponding to M_r 35000–45000 (β), M_r 25000–35000 (γ) and M_r 15000–25000 (a region lacking β or γ monomers or aggregates) excised. Antibody was eluted from the nitrocellulose strips in 0.1 M-glycine/HCl, pH 2.5, containing 0.15 M-NaCl and neutralized with unbuffered 0.5 M-Tris. The material eluted from each strip was incubated with fresh blots of normal- and Leach-phenotype-erythrocyte ghosts and bound antibody was detected by peroxidase labelled goat anti-human Ig. Tracks (*a*)–(*h*) are immunoblots of normal erythrocyte ghosts (*b,d,f,h*) or Leach-phenotype ghosts (*a,c,e,g*). Tracks (*a*) and (*b*) were incubated with unfractionated human anti-Ge (44017). Tracks (*c*) and (*d*) were incubated with the eluate from the β region of the nitrocellulose. Tracks (*e*) and (*f*) were incubated with the eluate from the γ region of the nitrocellulose. Tracks (*g*) and (*h*) were incubated with the eluate from the M_r-15000–25000 region of the nitrocellulose.

anti-β activity of the sera reacting with both β and γ is not dependent on the presence of an *N*-glycosidically linked oligosaccharide.

The abnormal sialoglycoproteins of Ge-negative erythrocytes have unusual *N*-glycans

We have previously reported (Reid *et al.*, 1985; Tanner *et al.*, 1987) that both β and γ are unaffected when intact erythrocytes are treated with endo-β-galactosidase and that treatment with Endo F results in a decrease in the M_r of a proportion of β molecules by about 3500 while γ remains unaffected. The observed decrease in the M_r of β on Endo F treatment is consistent with the known presence of an *N*-glycosidically linked oligosaccharide on this molecule (Dahr *et al.*, 1982). The resistance to endo-β-galactosidase suggests that repeated *N*-acetyl-lactosamine-containing units are absent from β.

The abnormal sialoglycoprotein components present in membranes from Ge-negative erythrocytes of the Ge type and the Yus type give much broader bands on SDS/polyacrylamide-gel electrophoresis than do normal β and γ (Anstee *et al.*, 1984*b*). The possibility that these broader bands result from heterogenous glycosylation was investigated by using endo-β-galactosidase and Endo F treatment of intact erythrocytes with subsequent SDS/polyacrylamide-gel electrophoresis on the isolated membranes and immunoblotting using a murine monoclonal anti-β antibody (BRIC 10) which has previously been shown to react with the abnormal components present in both these cell types (Anstee *et al.*, 1984*b*). When small amounts of ghosts were applied to the gels, the broad bands corresponding to the abnormal components of Ge-type and Yus-type Ge-negative cells were resolved into a series of distinct bands ranging from M_r 27000 to 35000 for Ge-type cells and M_r 29000–36000 for Yus-type cells (Fig. 3). After treatment with endo-β-galactosidase, the relative intensity of staining of these bands was altered. In Ge-type cells the leading edge of the band (M_r 27000–28000) was increased in staining intensity. In Yus-type cells the leading edge of the band (M_r 29000–30000) became more prominent. As expected, β of normal cells was unaffected by this treatment (Fig. 3*h*). These results suggest that polylactosamine groups are present on the abnormal sialoglycoprotein components and absent from normal β. When the cells were treated with Endo F, a new lower-M_r (24000–25500) band appeared in Ge-negative cells of the Ge type, whereas a new band of M_r 26000–27000 appeared in Ge-negative cells of the Yus type. Identical treatment of normal erythrocytes resulted in the expected decrease in the M_r of a proportion of the normal β band from M_r 36500–38000 to M_r 34000–34500 (Fig. 3). In order to exclude the possibility of a general abnormality in the *N*-glycosylation of cell-surface glycoproteins in Ge-negative erythrocytes, immunoblotting with murine monoclonal antibody R1.3 (which specifically binds α and δ; Tanner *et al.*, 1987) was used to show that, under the conditions used, Endo F decreased the M_r of α by about 3000 in normal erythrocytes and in all three Ge-negative phenotypes (Leach, Ge and Yus). This confirms that the *N*-glycosidically linked oligosaccharide of normal α is of similar size in normal and all three types of Ge-negative cells (results not shown). These results strongly suggest that the abnormal sialoglycoproteins found in Ge-negative erythrocytes of the Ge and Yus types have an increased content of *N*-glycosidically linked oligosaccharide(s). These abnormal sialoglycoproteins also contain a proportion of polylactosamine units, and it is likely that heterogeneity in the content of these units gives rise to the broad band found on gel electrophoresis. A similar heterogeneity in carbohydrate content is known to

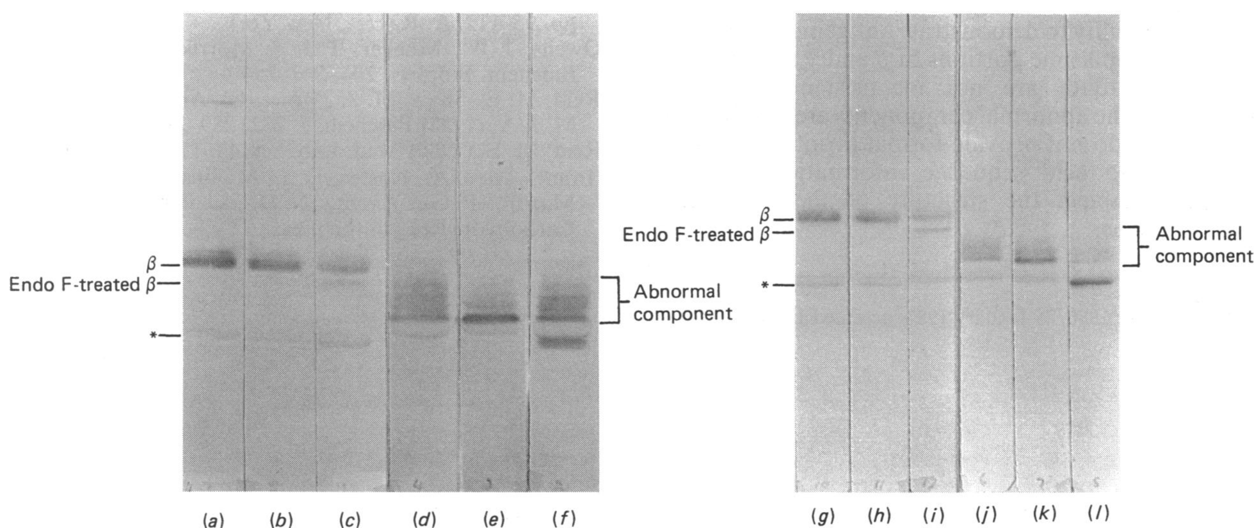

Fig. 3. Effect of endoglycosidases on β and the abnormal components of Ge-negative erythrocytes of the Ge type and the Yus type

The Figure shows immunoblots using monoclonal antibody BRIC 10. (*a*)–(*c*) and (*g*)–(*i*), Membranes from normal erythrocytes which were untreated (*a*,*g*), treated with endo-β-galactosidase (*f*,*h*) or treated with Endo F (*c*,*i*). (*d*)–(*f*), Membranes from Ge-negative erythrocytes of the Ge type (Ada; from L. A. McCall, American Red Cross, Tidewater, VA, U.S.A.), which were untreated (*d*), treated with endo-β-galactosidase (*e*) or treated with Endo F (*f*). (*j*)–(*l*) Membranes from Ge-negative erythrocytes of the Yus type (Cho) which were untreated (*j*) treated with endo-β-galactosidase (*k*) or treated with Endo F (*e*). (*a*)–(*f*) and (*g*)–(*l*) represent separate experiments. The band marked with an asterisk was found in all samples and probably represents non-specific binding (see Reid *et al.*, 1985).

contribute to the diffuse migration of erythrocyte band 3 on SDS/polyacrylamide gels (Mueller *et al.*, 1979).

Evidence is accumulating that the conformation of the polypeptide backbone influences oligosaccharide processing (reviewed by Kornfeld & Kornfeld, 1985). It is possible that this increased glycosylation of the abnormal components results from altered oligosaccharide processing, because of differences in the conformation of the polypeptides resulting from structural differences between these abnormal components and normal β.

In the present study we have used antibodies to probe the structural relationships between β, γ and the abnormal sialoglycoproteins found in some Ge-negative erythrocytes. The results presented here clearly show that portions of both the cytoplasmic and extracellular regions of β and γ are structurally related, since they share epitopes. However, the results also demonstrate that the extracellular regions of these molecules are antigenically different, since most human anti-Ge sera are specific for γ. Further, monoclonal anti-β antibodies (BRIC 4 and BRIC 10) do not react with γ, but do react with the abnormal sialoglycoprotein components of Ge- and Yus-type Ge-negative erythrocytes, demonstrating that the extracellular portions of these abnormal components are structurally related to β (Anstee *et al.*, 1984*b*; Figs. 2 and 3). Since the abnormal components do not react with most human anti-Ge sera, they lack the antigenic determinants present on the extracellular portion of γ which react with these antibodies. However, the reactivity of three anti-Ge sera with β, γ and the abnormal component of Ge-negative erythrocytes of the Yus type may indicate the presence of an extracellular epitope common to all these molecules. It is possible that these abnormal components are hybrid molecules with the *N*-terminus derived from β and the *C*-terminus derived from γ. β, γ and the abnormal components are all associated with the erythrocyte skeleton, and this may impose structural constraints on the cytoplasmic portions of these molecules which might make them antigenically similar. Our inability to demonstrate antigenic differences between the cytoplasmic portions of β and γ means that, although these data are not inconsistent with the hypothesis that the abnormal components are hybrid β–γ molecules, they do not provide formal proof that this is the case. Amino acid sequence information will be required to establish the structure of the abnormal sialoglycoproteins.

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REFERENCES

Anstee, D. J. & Tanner, M. J. A. (1974) Biochem. J. **138**, 381–386

Anstee, D. J., Mawby, W. J. & Tanner, M. J. A. (1979) Biochem. J. **183**, 193–203

Anstee, D. J., Parsons, S. F., Ridgwell, K., Tanner, M. J. A., Merry, A. H., Thomsen, E. E., Judson, P. A., Johnson, P., Bates, S. & Fraser, I. D. (1984*a*) Biochem. J. **218**, 615–619

Anstee, D. J., Ridgwell, K., Tanner, M. J. A., Daniels, G. L. & Parsons, S. F. (1984*b*) Biochem. J. **221**, 97–104

Dahr, W., Beyreuther, K., Kardowicz, M. & Kruger, J. (1982) Eur. J. Biochem. **125**, 57–62

Daniels, G. L., Shaw, M.-A., Judson, P. A., Reid, M. E., Anstee, D. J., Colpitts, P., Cornwall, S., Moore, B. P. L. & Lee, S. (1986) Vox Sang. **50**, 117–121

Dodge, J. T., Mitchell, C. & Hanahan, D. J. (1963) Arch. Biochem. Biophys. **100**, 119–130

Elder, J. H. & Alexander, S. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 4540–4544

Gahmberg, C. G. & Andersson, L. C. (1977) J. Biol. Chem. **212**, 5888–5894

Kornfeld, R. & Kornfeld, S. (1985) Annu. Rev. Biochem. **54**, 631–664

Laemmli, U. K. (1970) Nature (London) **227**, 680–682

Langone, J. T. (1982) J. Immunol. Methods **55**, 277–296

Mallinson, G., Martin, P. G., Anstee, D. J., Tanner, M. J. A., Merry, A. H., Tills, D. & Sonneborn, H. H. (1986) Biochem. J. **234**, 649–652

Mawby, W. J., Anstee, D. J. & Tanner, M. J. A. (1981) Nature (London) **291**, 161–162

Mueller, T. J., Li, Y.-T. & Morrison, M. (1979) J. Biol. Chem. **254**, 8103–8106

Mueller, T. J. & Morrison, M. (1981) in Erythrocyte Membranes:2: Recent Clinical and Experimental Advances, pp. 95–112, A. R. Liss, New York

Owens, J. W., Mueller, T. J. & Morrison, M. (1980) Arch. Biochem. Biophys. **204**, 247–254

Reid, M. E., Shaw, M.-A., Rowe, G., Anstee, D. J. & Tanner, M. J. A. (1985) Biochem. J. **232**, 289–291

Reid, M. E. (1986) Med. Lab. Sci. **43**, 177–182

Tanner, M. J. A., Anstee, D. J., Mallinson, G., Ridgwell, K., Martin, P. G., Avent, N. D. & Parsons, S. F. (1987) Carbohydr. Res., in the press