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Abnormal minor human erythrocyte membrane sialoglycoprotein (β) in association with the rare blood-group antigen Webb (Wb)

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1. Individuals whose erythrocytes are positive for the rare blood-group antigen Webb (Wb) have an altered form of the minor sialoglycoprotein β (synonyms glycophorin C and glycoconnectin). 2. This altered sialoglycoprotein β (β^{Wb}) has an M_r about 2700 lower than that of normal sialoglycoprotein β. 3. Treatment of normal sialoglycoprotein β with endo-β-*N*-acetylglucosaminidase F decreased its M_r by about 3600, but similar treatment of sialoglycoprotein β^{Wb} had no effect. 4. These results suggest the possibility that sialoglycoprotein β^{Wb} lacks the *N*-glycosidically linked oligosaccharide found on normal sialoglycoprotein β.

INTRODUCTION

The erythrocyte antigen Webb (Wb) is a low-incidence antigen initially described with a frequency of 0.06% (2 in 3550) in white Australians (Simmons & Albrey, 1963). The frequency is even lower, 0.02% (3 in 15815) in English blood donors (Cleghorn, 1967). *Wb* is inherited as a Mendelian dominant and has been shown to be independent of the following blood-group loci: *ABO*, *MNSs*, P^1, *Rh*, *K*, *Fy* and *Jk* (Simmons & Albrey, 1963; Cleghorn, 1967).

While examining erythrocyte membranes from individuals whose erythrocytes possess low-incidence blood-group antigens by SDS/polyacrylamide-gel electrophoresis, those erythrocytes positive for the Wb antigen were found to have an abnormal SGP β. A study of the abnormal SGP suggests that it probably lacks the *N*-glycosidically linked oligosaccharide normally found on β.

MATERIALS AND METHODS

A recent investigation to determine the frequency of Wb positive in Welsh blood donors revealed several propositi (L. Bloomfield, G. Rowe & C. A. Green, unpublished work). Erythrocytes from some of these donors were used in the present study. The ISBT Working Party on Terminology for Red Cell Surface Antigens has allocated the number 700009 to Wb (Lewis *et al.*, 1985).

Monoclonal antibodies BRIC 4 and BRIC 10 have been described previously (Anstee *et al.*, 1984*b*). Endo F was prepared from cultures of *Flavobacterium meningosepticum* as described by Elder & Alexander (1982). This preparation contains both Endo F and a peptidyl *N*-glycanase activity (Plummer *et al.*, 1984). *F. meningosepticum* was obtained from Dr. J. H. Elder, Research Institute of Scripps Clinic, La Jolla, CA 92037, U.S.A.

Treatment of erythrocytes with Endo F was as follows. Erythrocytes (100 μl of packed erythrocytes) were washed twice with 0.15 M-NaCl, then twice with 25 mM-sodium phosphate/0.1125 M-NaCl/0.0497 M-EDTA, pH 6.0. The final wash solution contained 2 mM-PMSF. After incubation with Endo F (10 μl) overnight at 37 °C in final wash solution, the erythrocytes were washed four times in 0.15 M-NaCl before serological tests or ghost preparation.

The methods for preparation of erythrocyte membranes and cytoskeletons, SDS/polyacrylamide-gel electrophoresis and periodate/NaB^3H_4 labelling of intact erythrocytes have been described previously (Anstee *et al.*, 1984*a*). Immunoblotting was carried out as described by Merry *et al.* (1985). Treatment of intact erythrocytes with neuraminidase and trypsin have been described previously (Anstee *et al.*, 1979; Tanner *et al.*, 1980).

Standard serological techniques were used. For indirect agglutination tests, anti-(human IgG)+anti-(human C3d) sera were used. Whole serum containing human anti-Wb was used for agglutination tests with trypsin-treated erythrocytes. For agglutination tests with neuraminidase-treated erythrocytes and Endo F-treated erythrocytes, human anti-Wb was prepared by absorption on to (room temperature for 1 h), and elution from (56 °C for 10 min), Wb-positive erythrocytes.

RESULTS AND DISCUSSION

SDS/polyacrylamide-gel electrophoresis of Wb-positive erythrocyte membranes with subsequent PAS staining revealed the presence of an abnormal component of M_r 36200 ± 140 ($n = 3$) and substantially decreased staining in the region of normal β-SGP compared with Wb-negative membranes (Figs. 1*a* and 1*b*). The abnormal component was observed in the membranes of seven Wb-positive donors not known to be related and six Wb-positive relations of five of the donors. All these individuals were proved by family studies to be heterozygous for Wb. Membranes from eight related

Abbreviations used: SDS, sodium dodecyl sulphate; PMSF, phenylmethanesulphonyl fluoride; Endo F, endo-β-*N*-acetylglucosaminidase F; SGP, sialoglycoprotein; PAS, periodic acid/Schiff's-base stain; β, sialoglycoprotein β.

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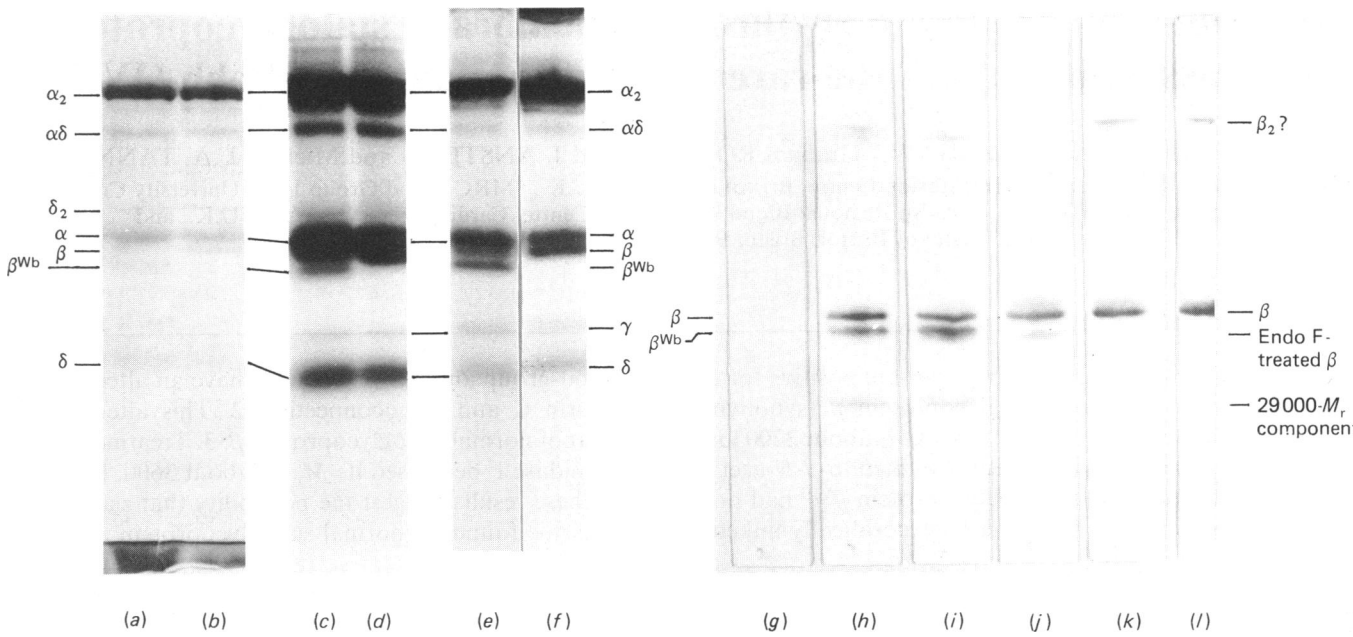

Fig. 1. SDS/polyacrylamide-gel electrophoresis of normal and Wb-positive erythrocyte membranes

(*a*) Wb-positive erythrocyte membranes stained with PAS; (*b*) normal erythrocyte membranes stained with PAS; (*c*) fluorograph of Wb-positive erythrocyte membranes labelled with periodate/NaB^3H_4 (5 days exposure); (*d*) fluorograph of normal erythrocyte membranes labelled with NaB^3H_4 (5 days exposure); (*e*) fluorograph of Wb-positive erythrocyte cytoskeletons labelled with NaB^3H_4 ($2\frac{1}{2}$ days exposure); (*f*) fluorograph of normal erythrocyte cytoskeletons labelled with NaB^3H_4 (5 days exposure). Tracks *g–l*, immunoblots with monoclonal antibody BRIC 10: (*g*) membranes from a donor with erythrocytes of Leach phenotype (donor S.S.); (*h*) membranes from Wb-positive erythrocytes (donor M.E.); (*i*) membranes from Endo F-treated Wb-positive erythrocytes (donor M.E.); (*j*) membranes from Endo F-treated normal erythrocytes (donor W.E.); (*k*) membranes from normal erythrocytes (donor W.E.); (*l*) membranes from normal erythrocytes. The samples in tracks (*h*) and (*k*) were incubated at pH 6.0 under the same conditions as the Endo F-treated samples in tracks (*i*) and (*j*).

Wb-negative members of four of the familes showed normal SGP profiles. When the separated erythrocyte membrane components were stained with Coomassie Blue, no differences from normal were observed (results not shown). When the sialic acid-containing membrane glycoproteins of Wb-positive erythrocytes were labelled by using the periodate/NaB^3H_4 method, the abnormal PAS-staining component was clearly labelled (Figs. 1*c* and 1*d*). The abnormal component was also present in cytoskeletons prepared from periodate/NaB^3H_4-labelled Wb-positive erythrocytes, as was the apparently normal β (Figs. 1*e* and 1*f*). Normal β is known to remain associated with the erythrocyte skeleton (Mueller & Morrison, 1981).

The reduction in the PAS staining of normal β in Wb-positive erythrocyte membranes suggested the possibility that the abnormal component was an altered β-SGP. This was confirmed by using two mouse monoclonal antibodies (BRIC 4 and BRIC 10) specific for normal β-SGP [M_r $39100 \pm 700(11)$]. Immunoblotting using these monoclonal antibodies showed that they reacted with the abnormal component of Wb-positive erythrocytes. The abnormal component had the same M_r as that observed when the PAS stain was used [M_r $36400 \pm 200(4)$] (data for BRIC 10 shown in Fig. 1*h*).

Normal β is known to contain an *N*-glycosidically linked oligosaccharide (Dahr *et al.*, 1982). The substantial difference in M_r between normal β and the abnormal β component of Wb-positive erythrocytes (~ 2700) suggested that the *N*-glycosidically linked oligosaccharide was not present on the abnormal β component. Absence of such an oligosaccharide occurring together with the appearance of rare blood-group antigen has already been described for SGP α (synonym glycophorin A), where lack of the *N*-glycosidically linked oligosaccharide is associated with the occurrence of the rare blood-group antigens Vw and Hut (Dahr *et al.*, 1984). Endo F preparations can be used to cleave *N*-glycosidically linked oligosaccharides from glycoprotein (Elder & Alexander, 1982). SDS/polyacrylamide-gel electrophoresis of Endo F-treated normal erythrocyte membranes with subsequent immunoblotting using mouse monoclonal anti-β (BRIC 4 or BRIC 10) results in the appearance of a band of M_r $35500 \pm 400(2)$ (data for BRIC 10 in Fig. 1*j*). Normal β was found to be relatively resistant to Endo F treatment (Fig. 1*j*). By using the available Endo F preparations, onoly a partial cleavage could be obtained, even under conditions of overnight incubation. Under similar conditions, the *N*-glycosidically linked oligosaccharide of SGP α was completely cleaved in 3 h (results not shown).

A band of M_r $75000 \pm 500(11)$, which may correspond to a dimer of normal *B*, was observed on immunoblotting of normal membrane preparations. There was a decrease in staining of this putative dimer band after treatment with Endo F and the appearance of a new band of lower M_r (Figs. 1*j* and 1*k*). When Endo F-treated Wb-positive erythrocytes were analysed, a band corresponding to Endo F-treated normal β was present in addition to the abnormal Wb-positive component. No additional new band was observed, suggesting that either the abnormal β of Wb-positive erythrocytes lacks an *N*-glycosidically linked oligosaccharide or that it is unusually resistant to

Endo F treatment (Fig. 1*i*). No additional new band was obtained on immunoblots of Endo F-treated Wb-positive erythrocyte membranes when the same samples were applied at three times the loading shown in Figs. 1*h*–1*k*) (results not shown). The abnormal β in Wb-positive erythrocytes had a higher (~ 900) M_r than had the Endo F-treated product of normal β. The band of M_r 29000 ± 400(8) was present under the incubation conditions used whether Endo F was present or not (Fig. 1*h*–1*k*). The binding of BRIC 10 to this M_r-29000 component was non-specific, since monoclonal antibodies directed at different erythrocyte membrane components also bound to this component (results not shown).

The binding of BRIC 10 to the abnormal β of Wb-positive erythrocytes was eliminated if the erythrocytes were first treated with either neuraminidase or trypsin. The binding of Bric 10) to normal β was also eliminated by such treatment (Anstee *et al.*, 1984*b*). The reactivity of anti-Wb with Wb-positive erythrocytes was eliminated if the erythrocytes had been treated with either neuraminidase or trypsin, but treatment with Endo F had no effect. The Wb antigen is not a cryptantigen on normal erythrocytes, since anti-Wb failed to react with Endo F-treated Wb-negative erythrocytes. These results are consistent with the location of the Wb antigen on the abnormal β-SGP, which we denote β^{Wb}.

The difference in M_r between β^{Wb} and the Endo F-treated product of normal β suggests that, if the *N*-glycosidically linked oligosaccharide is missing from β^{Wb}, there is a further alteration in the structure of β^{Wb}. The increased M_r of β^{Wb} (900) raises the possibility of an additional *O*-glycosidically linked sialotetrasaccharide (M_r 1000) on this molecule. An alteration in the polypeptide sequence could cause the loss of the *N*-glycosidically linked oligosaccharide and the concomitant generation of a new *O*-glycosylation site.

While this work was in progress we became aware of a similar study by Macdonald & Gerris (1985).

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