The W_r^b antigen, a receptor for *Plasmodium falciparum* malaria, is located on a helical region of the major membrane sialoglycoprotein of human red blood cells

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1. Immunoprecipitation of periodate/ NaB^3H_4 -labelled human erythrocytes using anti-Wright^b (W_r^b) monoclonal antibodies showed that these antibodies specifically react with the major erythrocyte sialoglycoprotein α (glycophorin A). 2. Similar experiments on erythrocytes from the only known individual lacking the W_r^b antigen but with otherwise normal sialoglycoproteins did not result in the immunoprecipitation of any sialoglycoprotein. 3. We suggest that the W_r^b antigen is located on an α -helical region between residues 55 and 70 of sialoglycoprotein α .

Invasion of red cells by malarial parasites depends, at least in part, on specific recognition between the merozoite form of the parasite and the red-cell surface. The different species of malarial parasites show a high degree of host specificity. *Plasmodium vivax* and *P. falciparum*, two human malarial parasites, recognize different features of the human red-cell surface. The receptor for *P. vivax* appears to involve the erythrocyte Duffy [Fy(a+) and Fy(b+)] blood-group antigens (Miller, 1977), whereas there is evidence that the major erythrocyte sialoglycoproteins are involved in the invasion of erythrocytes by *P. falciparum*. Soluble sialoglycoprotein preparations inhibit invasion by this parasite (Perkins, 1981), and erythrocyte variants that lack the major sialoglycoprotein α [synonym glycophorin A; see Anstee *et al.* (1979) for nomenclature] are relatively resistant to invasion (Pasvol *et al.*, 1982a). Variants which lack sialoglycoprotein δ (synonym glycophorin B) are also more resistant to invasion than normal erythrocytes (Pasvol *et al.*, 1982b).

Further analysis has suggested that *P. falciparum* invasion requires at least two distinct receptors (Pasvol *et al.*, 1982b). One receptor appears to involve oligosaccharides associated with sialoglycoproteins α and δ , whereas the other receptor appears to involve the erythrocyte antigen W_r^b (Wright). Monoclonal anti- W_r^b antibodies are able to block *P.*

falciparum invasion of normal erythrocytes. In addition, erythrocytes which lack the W_r^b antigen (Adams *et al.*, 1971) but have otherwise apparently normal sialoglycoproteins are highly refractory to invasion (Pasvol *et al.*, 1982b).

The nature of the erythrocyte component carrying the W_r^b antigen is unknown. In the present paper we show that this antigen is located on sialoglycoprotein α , in a helical region of the protein close to the point where it enters the membrane.

Materials and methods

Monoclonal antibodies were produced by fusing spleen cells from mice immunized with intact human erythrocytes with the mouse myeloma NS1 as described by Parsons *et al.* (1982) and were used in all experiments as unprocessed ascitic fluid.

Three monoclonal antibodies with anti- W_r^b specificity (NBTS/BRIC 13, 14, 15) were used in the present study. The binding affinities of the monoclonal antibodies were assessed by competition experiments using normal erythrocyte membranes solubilized with 1% sodium deoxycholate (Maybridge Chemical Co., Tintagel, Cornwall, U.K.). Erythrocyte membranes were prepared from normal human red cells by lysis in 5 mM-sodium phosphate, pH 8.0, as described by Moore *et al.* (1982). Membranes were solubilized at 4°C with 4 vol. of 150 mM-NaCl/50 mM-Tris/HCl (pH 8.0)/1% (w/v) sodium deoxycholate, followed by centrifugation at 4°C for 1 h at 35 000g to remove any insoluble material. Supernatant (1 vol.) and antibody (1 vol.)

Abbreviations used: NBTS/BRIC, National Blood Transfusion Service/Bristol Immunochemistry; (α - δ)^{MIV}, the hybrid sialoglycoprotein present in Miltenberger class V erythrocytes.

were incubated for 30 min at 37°C, and the resulting titre of the antibody against normal human red cells was measured by haemagglutination in an indirect antiglobulin test using rabbit anti-mouse immunoglobulin G. The antibodies most inhibited by solubilized membranes were presumed to have the highest affinity (Mason & Williams, 1980).

Labelling of intact erythrocytes with sodium metaperiodate and NaB^3H_4 (Amersham International; sp. radioactivity 8.5 Ci/mmol) was by the method of Gahmberg & Andersson (1977). Immunoprecipitation of radiolabelled components from Triton X-100-solubilized erythrocyte ghosts was performed as described by Moore *et al.* (1982), except that an additional incubation (37°C, 30 min) with 50 μl of rabbit anti-mouse immunoglobulin G was included before the isolation of ghosts. The monoclonal antibodies most readily inhibited by deoxycholate-solubilized membranes were also found to be most effective in immunoprecipitation from Triton X-100 solubilized membranes. Wr(b—)

erythrocytes (Adams *et al.*, 1971) were obtained from the Paul I. Hoxworth Blood Center, Cincinnati, OH, U.S.A.

Washed Protein A-Sepharose beads (Pharmacia, Hounslow, Middx., U.K.), carrying adsorbed immune complexes, were resuspended in 100 μl of electrophoresis sample buffer [5% (w/v) sodium dodecyl sulphate/5 mM-EDTA/Tris (pH 8.0)/10% (v/v) glycerol/Bromophenol Blue (0.1 mg/ml)/Xylene Cyanol (0.1 mg/ml)/2 mM-phenylmethanesulphonyl fluoride/5% (v/v) β -mercaptoethanol], mixed, heated at 100°C for 2 min and the supernatants submitted to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis by the method of Laemmli (1970), on 7.5–15% (w/v) acrylamide gradients with 5% stacking gels. The gels were fixed in methanol/acetic acid/water (5:1:5, by vol.) for 30 min at 65°C, washed in water and soaked in 1 M-sodium salicylate (Chamberlain, 1979) for 30 min before drying and exposure to Kodak XAR-5 film at –70°C.

Fig. 1. Immunoprecipitation of membrane components from periodate/ NaB^3H_4 -labelled intact normal and Wr(b—) M.Fr. erythrocytes

(a–f) Autoradiographs of: (a) solubilized normal erythrocyte membranes; (b) components from ^3H -labelled normal erythrocytes precipitated by monoclonal antibody R18; (c) components from ^3H -labelled normal erythrocytes precipitated by monoclonal antibody BRIC 14; (d) components from ^3H -labelled Wr(b—) erythrocytes precipitated by monoclonal antibody R18; (e) components from ^3H -labelled Wr(b—) erythrocytes precipitated by monoclonal antibody BRIC 14; (f) solubilized Wr(b—) erythrocyte membranes. Autoradiographs (a)–(c) and (d)–(f) are from different experiments, therefore the bands have slightly different mobilities.

The sialic acid content of erythrocytes was determined as described by Anstee *et al.* (1979).

Results

The monoclonal antibodies NBTS/BRIC 13, 14 and 15 were shown to have anti-Wr^b specificity by their reaction with all normal human erythrocytes but not with the Wr(b-) erythrocytes of donor M.Fr. Their specificity is the same as that of monoclonal antibody R7 (Anstee & Edwards, 1982). All the monoclonal anti-Wr^b antibodies were tested for their susceptibility to inhibition by deoxycholate-solubilized normal red-blood-cell membranes by using haemagglutination inhibition. BRIC 14 was most readily inhibited under these conditions and was therefore assumed to have the highest binding affinity.

Immunoprecipitation of membrane components from periodate/NaB³H₄-labelled intact normal erythrocytes gave the results shown in Fig. 1. BRIC 14 clearly precipitates the major sialoglycoprotein α and its dimer, α_2 . Similar results (not shown) were obtained with BRIC 15. Parallel control experiments using monoclonal antibody R18 [with known anti-(sialoglycoprotein α) specificity; Edwards (1980)] confirmed that the bands precipitated by BRIC 14 have the same mobility as sialoglycoprotein α (Fig. 1).

All the known erythrocytes lacking sialoglycoprotein α also lack the Wr^b antigen (Anstee, 1981), as do erythrocytes carrying hybrid α - δ sialoglycoproteins. In contrast, the Wr(b-) cells of M.Fr. have a normal sialic acid content ($101 \pm 0\%$ of normal erythrocytes; four determinations) and the pattern of staining of the membranes with periodic acid/Schiff's-base stain after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis is identical with that of normal membranes (results not shown). Additional evidence that portions of the sialoglycoprotein α polypeptide chain in M.Fr. cells are the same as normal sialoglycoprotein α is provided by the observation that M.Fr. cells react with two monoclonal antibodies (R10, R18) directed against different portions of sialoglycoprotein α (Anstee & Edwards, 1982). The Wr(b-) cells of M.Fr. are unique in having otherwise apparently normal sialoglycoproteins. In order to confirm that the Wr^b antigen is located on sialoglycoprotein α , immunoprecipitation was also done with the Wr(b-) M.Fr. erythrocytes. Immunoprecipitation with periodate/NaB³H₄-labelled M.Fr. cells (Fig. 1) showed that BRIC 14 did not precipitate sialoglycoprotein α from these cells under conditions in which sialoglycoprotein α was clearly immunoprecipitated with the monoclonal antibody R18.

Discussion

The present results show that the Wr^b antigen is located on sialoglycoprotein α . The occurrence of

secondary changes at the surface of erythrocytes lacking sialoglycoprotein α (e.g. altered glycosylation of the anion-transport protein; Tanner *et al.*, 1976) and the failure of soluble sialoglycoprotein α preparations to inhibit anti-Wr^b antibodies (Anstee & Edwards, 1982) has until now prevented the unequivocal assignment of the Wr^b antigen to sialoglycoprotein α . The portion of sialoglycoprotein α in which the Wr^b antigen is located can be inferred by the absence of the Wr^b antigen from variant erythrocytes containing hybrid sialoglycoproteins with the N-terminal portion of sialoglycoprotein α and C-terminal portion of sialoglycoprotein δ [e.g. (α - δ)^{MIV}; see Anstee *et al.* (1982a) for a review]. These hybrid sialoglycoproteins appear to lack the portion of sialoglycoprotein α on the C-terminal side of residue 55. The Wr^b antigen is therefore most likely to be located between residues 56 and 72 (where the polypeptide chain enters the

Fig. 2. Secondary-structure prediction of the Wr^b-antigen-containing portion of sialoglycoprotein α .

The amino acid sequence of sialoglycoprotein α (Tomita *et al.*, 1978) was used to carry out secondary-structure prediction using the computer program described by McLachlan (1977). The ordinate gives the probability of finding a given secondary structure at a particular residue. 0 = α -helix; I = β -sheet; $\star$ = bend. The amino acid residues [denoted by the single-letter amino acid code; see *Biochem. J.* (1969) 113, 1-4] are indicated at the top of the diagram and the residue number on the abscissa. No glycosylated residues are present between residue 51 and the C-terminus of the molecule. The helical region between residues 55 and 69 was the most strongly predicted region of secondary structure (had the highest predicted probability) in the entire sequence of sialoglycoprotein α . The membrane-penetrating region of the protein extends from residues 73 to 92 (Tomita *et al.*, 1978). Although this region is thought to have α -helical structure (Schulte & Marchesi, 1979), the program predicts it as β -sheet structure. The location of this helix in a hydrophobic environment probably gives rise to this anomaly.

lipid bilayer). This assignment is consistent with the known retention of the W_r^b antigen on erythrocytes that have been treated with proteolytic enzymes (Issitt *et al.*, 1976).

Secondary-structure prediction of sialoglycoprotein α was carried out by using the known sequence determined by Tomita *et al.* (1978) and the computer program described by McLachlan (1977). Fig. 2 shows that between two regions of bend (residues 53–54 and 70–72) is a segment of polypeptide which is very strongly predicted to be α -helical. No glycosylated residues are present in this region. This α -helical region corresponds exactly with the predicted location of the W_r^b antigen discussed above and contains a high density of charged residues. Hopp & Woods (1981) have reported that the most highly charged regions of amino acid sequences form the strongest antigenic sites on other proteins. It is possible that the predicted α -helical segment carrying the W_r^b antigen forms an independent domain linking the *N*-terminal extracellular portion of the protein to the membrane.

The red-cell antigens W_r^a and W_r^b have been considered to be the product of allelic genes, since the only known $W_r(b-)$ negative donor (M.Fr.) also has the relatively rare W_r^a antigen (Adams *et al.*, 1971). Sialoglycoprotein α carries the blood-group MN antigen, and it is established that the W_r^a antigen is inherited independently of the blood-group MNSs system (Race & Sanger, 1975) and therefore independently of sialoglycoprotein α . Our results clearly imply that the genes giving rise to W_r^a and W_r^b antigens are not allelic. The naming of the W_r^b antigen is therefore rather inappropriate.

From these results we can infer the nature of the change in sialoglycoprotein α of M.Fr. cells ($\alpha^{M.Fr.}$) which results in the loss of the W_r^b antigen. There is no detectable difference in the electrophoretic mobility of $\alpha^{M.Fr.}$ and normal sialoglycoprotein α , and therefore it is unlikely that there is any extensive deletion in $\alpha^{M.Fr.}$. Since $\alpha^{M.Fr.}$ is reactive with monoclonal antibodies R10 and R18 (Anstee & Edwards, 1982), it also retains the characteristics of sialoglycoprotein α from the *N*-terminus to residue 55 (the determinants for R10 and R18 are located in this region; Anstee *et al.*, 1982*a,b*). Thus the change must be localized to the helical region between residues 56 and 72 and is likely to be a point mutation (perhaps affecting a charged residue in this region), which results in the loss of the W_r^b antigen.

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