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Receptors on red cells for *Plasmodium falciparum* and their interaction with merozoites

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The red cell sialoglycoproteins (glycophorins, α(A), δ(B) and β and γ(C)) play a crucial role in the invasion of human red cells by merozoites of *Plasmodium falciparum*. Red cells deficient in any of the glycophorins, including β (also known as glycoconnectin), resist infection by this parasite to varying degrees. These cells and other naturally occurring well-characterized glycophorin variants provide extremely powerful tools to dissect the role of these molecules in invasion. The binding of merozoites to human red cells appears analogous to the binding of wheatgerm agglutinin to sialoglycoconjugates. In both systems O- and N-linked oligosaccharides may be involved. Membrane lipid has not been implicated as a receptor for merozoites, but may instead non-specifically modify binding, as may electrostatic and hydrophobic interactions. The results of data using monoclonal antibodies and lectins, although possibly helpful in identifying specific determinants, must be interpreted with caution. Overall the data suggest that the red cell receptors for all strains of *P. falciparum* tested to date are located on the glycophorins. Accordingly these putative receptors have been used to affinity-purify complementary parasite components which may yet prove to be of protective immunological significance in a vaccine.

1. Introduction

During its complex life cycle in the vertebrate host, the malarial parasite must enter the red cell. This is a highly specific and ordered process in which the invasive form or merozoite makes contact with, recognizes and attaches to a susceptible red cell; orients its apical end to the red cell surface; forms a junction between itself and red cell; and finally enters the cell by means of an invagination in the red cell membrane. This gives rise to the erythrocytic part of the parasite cycle responsible for the clinical manifestations of the disease which, in the case of *Plasmodium falciparum* infections in man has a high mortality worldwide. There is now substantial evidence that the merozoites of each plasmodial species attach to specific receptors on the red cell surface of the susceptible host during invasion (reviewed by Howard & Miller 1981; Pasvol & Wilson 1982). Because of the apparent specificity and the functional importance of this interaction, it might be expected that these merozoite components essential for invasion would be highly conserved and less likely to undergo antigenic variation. Isolated putative red cell receptors can be used to probe for complementary merozoite antigens. This has provided a rational and novel strategy in the search for potential plasmodial immunogens.

2. Red cell receptors

The role of the major glycophorins, α(A) *and* δ(B)

The suggestion that the red cell membrane sialoglycoproteins or glycophorins (for a review of these molecules see Anstee (1981) and figure 1) might serve as receptors for *P. falciparum* came from Miller *et al.* (1977) who showed that the red cells of two rare individuals which lack α (glycophorin A) were relatively resistant to infection by this parasite.

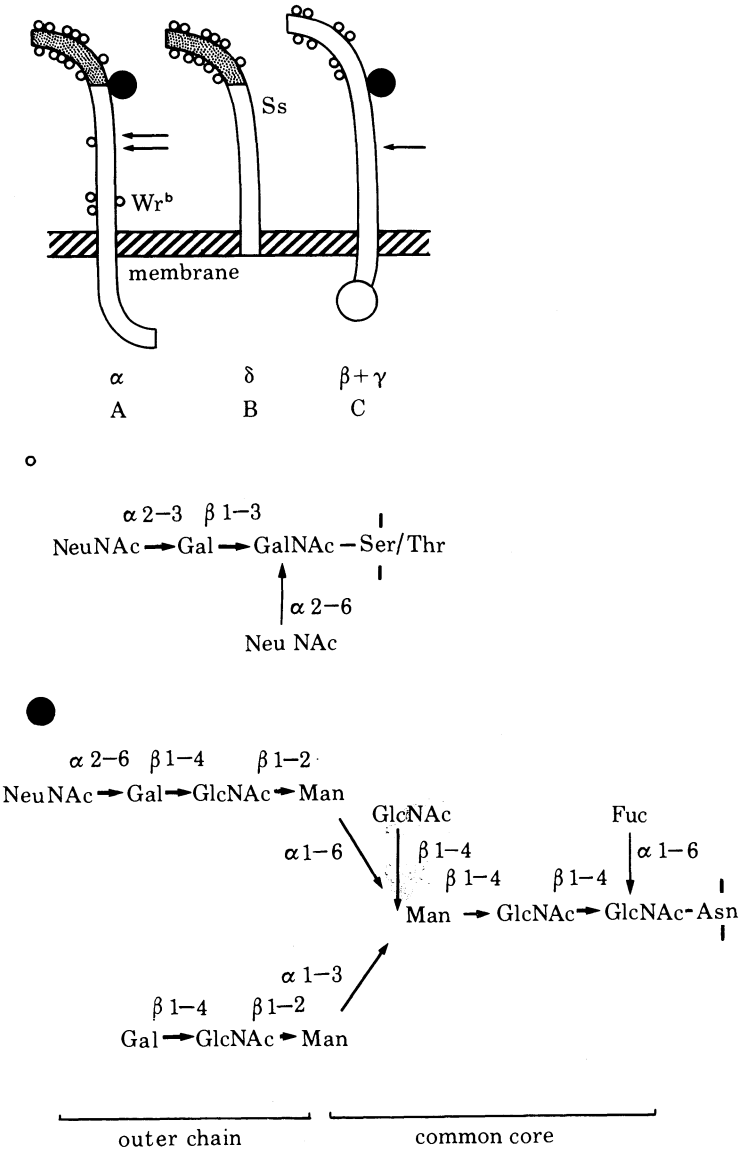

Figure 1. A schematic representation of the red cell sialoglycoproteins or glycophorins: α(A), δ(B), and β and γ(C) (Anstee 1981). The shaded areas of α and δ indicate regions of homology; the arrows indicate trypsin cleavage sites on intact cells. β is thought to be linked to the underlying cytoskeleton via band 4.1. The small circles represent *O*-glycosidically linked oligosaccharides and the large shaded circles represent *N*-glycosidically linked oligosaccharides present on α, β and γ but not δ. The location of the antigenic determinants Wright^b (Wr^b) and Ss are indicated. The structures of the oligosaccharides are also shown. The abbreviations are: NeuNAc, *N*-acetylneuraminic acid; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Ser, serine; Thr, threonine; GlcNAc, *N*-acetylglucosamine; Man, mannose; Fuc, fucose; Asn, asparagine.

These results were later confirmed with red cells from three such individuals (Pasvol *et al.* 1982*a*). Moreover, it was shown that the few parasites that entered these glycophorin-deficient cells matured normally. In addition, sera from these α-deficient individuals inhibited merozoite invasion of normal cells. These sera possessed antibodies of at least three specificities, namely, against the trypsin-sensitive and trypsin-insensitive portions of α respectively, and against another determinant called Wright^b (Wr^b) which has since been found to be located on α close to the membrane (Ridgwell *et al.* 1983). Antibodies against other commonly occurring red cell antigens such as rhesus (C and c), Kidd (Jk^a) and Duffy (Fy^a) were without effect. Thus it appeared that the role of α was mainly in the invasion process and that this was to some degree specific.

The limited invasion of cells deficient in α implies that alternative means must exist for parasite invasion in the absence of this molecule. Individuals with cells deficient in δ (glycophorin B) were also relatively resistant to invasion in some studies (Pasvol *et al.* 1982*b*; Facer 1983) but not in others (Howard *et al.* 1982). However, trypsin treatment, which removes most of the remaining membrane sialoglycoproteins (α, β and γ) of these δ-deficient cells, reduced invasion to almost negligible levels in all three studies. Cells possessing a hybrid glycophorin molecule with the amino-terminal end of α (including the trypsin-sensitive site) and carboxy-terminal end of δ were also resistant to invasion (54% inhibition) which was increased even further on trypsin treatment (Pasvol *et al.* 1982*b*). These results indicate that both α and δ are important for merozoite invasion of red cells (Pasvol & Jungery 1983).

The red cell sialoglycoproteins have also been tested in solution for their ability to inhibit invasion by *P. falciparum* merozoites *in vitro*. Results of these studies have differed widely, highlighting the value of using naturally occurring glycophorin-deficient cells. Weiss *et al.* (1981) for example did not implicate the sialoglycoproteins since concentrations of 0.5 mg ml^{-1} resulted in only 28% inhibition, whereas Perkins (1981) found that purified α abolished invasion at concentrations as low as 50 μg ml^{-1}. Deas & Lee (1981) found that about 0.25 mg ml^{-1} of glycophorin was required for 50% inhibition whereas Jungery *et al.* (1983*a*) used concentrations of approximately 1 mg ml^{-1} and more recently Breuer *et al.* (1983*a*) required 0.1 mg ml to achieve the same inhibition. The variability of these data emphasize the difficulty in using solubilized glycophorins. When inserted into liposomes inhibition by α was increased sixfold (Breuer *et al.* 1983*a*). Interestingly these studies have demonstrated some species specificity in that sheep glycophorins had no effect on invasion (Perkins 1981; Deas & Lee 1981) whereas glycophorin from rhesus monkeys showed some invasion inhibition of *P. falciparum* into human red cells, for example, 50 μg ml^{-1} was required for 50% inhibition (Perkins 1981).

The role of β *and* γ *(glycophorin C)*

The β and γ glycophorins (glycophorin C) make up only a minor proportion of the red cell sialoglycoproteins (approximately 10%); β is, however, the only known sialoglycoprotein that is directly linked to the underlying red cell cytoskeleton to band 4.1 and has been named glycoconnectin (Mueller & Morrison 1981). The commonly occurring Gerbich antigen might possibly be located on β or γ (Anstee *et al.* 1984*b*). The recent discovery of a patient (P.L.) whose red cells are deficient in β and γ (Anstee *et al.* 1984*a*), and the use of trypsin treatment of α-deficient cells which removes these two minor sialoglycoproteins, has facilitated an investigation into the role of β and γ in invasion. The results showed that parasites were still capable

of entering these glycoconnectin-deficient cells although in reduced numbers (57% of the controls; table 1).

TABLE 1. INVASION OF RED CELL MEMBRANE VARIANTS BY *P. FALCIPARUM*

			percentage of control		
cells	molecular defect	control (rings per 100 red cells)	control trypsin-treated	variant	variant trypsin-treated
PL	β and γ-deficient	13.0±1.3	34±3	57±4	22±4
LC	α-deficient (En(a−))	9.8	55	39	22
Gerbich−ve	hybrid β-γ	7.3±1.4	35±12	93±8	33±9
ovalocytic (Melanesian)	unknown	10.3	n.d.	8	n.d.
elliptocytic	unknown	10.7±0.7	34	96±2	31

Invasion of various red cell membrane or cytoskeletal variants by *Plasmodium falciparum*. Schizonts concentrated by gelatin were incubated with variant red cells for 12–18 h after which smears were made, stained and counted. Each result is indicated with the s.e.m. except with the cells of LC and the ovalocytic cells where the results are the means of two experiments. n.d., Not done.

Trypsin treatment of α-deficient cells, which removes β and γ (but leaves the membrane–cytoskeletal link intact), also reduced invasion further (table 1) confirming a minor role for the outer portions of β and γ in invasion. Cells with only δ remaining on their surface (that is, trypsin-treated normal, β- and γ-deficient, and α-deficient cells) were still invaded, emphasizing the importance of this sialoglycoprotein (δ) in its own right. It has however been suggested that invasion of trypsin-treated cells may in part be due to α-acid glycoprotein (AGP) in human serum which binds onto these cells and forms a bridge between merozoite and red cell (Friedman *et al.* 1984). The overall results however indicate a minor role for the outer portion of β and γ in invasion and that the direct link of β with the underlying cytoskeleton is not critical in the invasion of human red cells by *Plasmodium falciparum*.

The number of individuals with red cells negative for the Gerbich antigen reach an extremely high frequency in malarious areas such as Melanesia (Booth & McLoughlin 1972). Although these cells lack β and γ on periodic acid-Schiff (PAS)-stained gels, they appear to possess a hybrid molecule of intermediate molecular mass instead (Anstee *et al.* 1984*b*). When non-ovalocytic, Gerbich-negative cells from Papua New Guinea were tested, only a minor reduction in invasion was observed, in contrast to the β- and γ-deficient cells of PL and the almost total invasion inhibition seen with ovalocytic cells taken from individuals living in the same area (table 1). Invasion of another elliptocytic cell type taken from patients with the sporadically-occurring form of elliptocytosis, was normal.

The role of carbohydrate

The first indication that sugars may serve as specific determinants in the invasion of red cells by merozoites came from the findings of Miller *et al.* (1977) who found that neuraminidase treatment of human red cells reduced their susceptibility to invasion by *P. falciparum*, but not *P. knowlesi*. Subsequently a number of red cells with modified oligosaccharides located on the glycophorins have been found to resist infection. For example, Tn cells, whose O-linked tetrasaccharides are deficient in the outer two sugars *N*-acetylneuraminic acid (NeuNAc) and galactose (Gal) attached to each other via an α 2–3 linkage (Anstee 1981) were found to be

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almost totally resistant (Pasvol *et al.* 1982*b*; Cartron *et al.* 1983; Perkins 1984). Cad cells, which possess an *N*-acetylgalactosamine (GalNAc) residue attached to the galactose of each O-linked tetrasaccharide via a β (1–4) linkage (Blanchard *et al.* 1983), were also found to reduce parasite multiplication (Cartron *et al.* 1983).

The importance of the oligosaccharide-rich outer portion of α in parasite invasion has recently been emphasized by Perkins (1984). The T1 fragment of the amino-terminal end of α (residues 1–39) obtained by trypsin treatment of the isolated molecule and which contains 13 of the 16 oligosaccharides present on α, was found to inhibit merozoite invasion by about 76% at a concentration of 100 μg ml^{-1}. The outermost portion of this fragment includes the well-characterized M–N antigen system defined by a sequence of five amino acids (residues 1–5) and three associated tetrasaccharides. However this portion on its own appears not to be involved in invasion. Cells expressing the M or N antigen or both were equally invaded (Pasvol *et al.* 1982*a*). Moreover, Cartron *et al.* (1983) described Mg Mg cells (which because of an amino acid substitution at position 4 have a defect in the sugar residues attached to amino acids 2, 3 and 4) and McM cells (which have an amino acid substitution at position 1 or 5) as being fully susceptible to infection. These data taken together would appear to exclude a definitive role for this small portion of the molecule. We have suggested that the binding of merozoite to red cell might involve a cluster rather than a single or few oligosaccharides for effective binding (Pasvol *et al.* 1982*b*). The most important cluster of sugars thus remaining on this trypsin-sensitive fragment are those O-linked oligosaccharides attached to amino acids 10–15 and the N-linked complex sugar at position 26.

Sugars, mainly monosaccharides have been used in solution to determine their effect on invasion. Of the sugars located on the O-linked oligosaccharides (figure 1) NeuNAc and GalNAc were found to be inhibitory but only at relatively high concentrations (more than 20 mM) (Jungery *et al.* 1983*a*). More recently inhibition has been observed with the α-and β-methylglycosidic derivatives of GalNAc (Hermentin *et al.* 1983). The disaccharide galactose Gal (β1–3) GalNAc, otherwise known as the 'T' hapten or tumour-associated disaccharide found on the O-linked sugars of the glycophorins, was even more inhibitory (Hermentin *et al.* 1984*a*).

Unfortunately the use in soluble form of the sugars located on the N-linked oligosaccharides to explore their role in invasion is problematical because of the difficulty in distinguishing invasion inhibition from toxic effects, especially on the maturing schizont. This has occurred particularly in the case of *N*-acetylglucosamine (GlcNAc) of which there are five molecules per N-linked sugar (figure 1) and where varying results have been obtained (Weiss *et al.* 1981; Howard *et al.* 1982; Jungery *et al.* 1983*a*; Perkins 1984). Whatever the mechanism of action of GlcNAc, the effect appears to be relatively specific and its presence at concentrations of the order of 10–25 mM leads to marked reduction in the multiplication of the parasite *in vitro*. It may well be that the effects of invasion inhibition and toxicity overlap partly. Only one of these studies (Jungery *et al.* 1983*a*) has attempted to exclude toxic effects of GlcNAc adequately. By coupling an aromatic (*p*-aminophenyl) derivative of the sugar to bovine serum albumin (BSA) it was not only shown that GlcNAc was inhibitory, but that presented to the parasite in this way, it was about 10^5-fold more potent than in solution; 10^{-4} compared with 20 mM being necessary for 70% inhibition. Lactose coupled to albumin in a similar fashion showed no inhibition at comparable concentrations. Recently Hermentin *et al.* (1983) have reported that the α and β methyl (Me) glycosides of GlcNAc and GalNAc are inhibitory without

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being toxic. In further studies (Hermentin *et al.* 1984*b*) GlcNAc was β-glycosidically coupled to BSA via an aliphatic [—$(CH_2)_8CO$—] and an amide spacer [—$(CH_2)_2NCHO(CH_2)_2CO$—] and less invasion inhibition was found than that observed by Jungery *et al.* (1983*a*). The *p*-nitrophenyl derivatives of GlcNAc and glucose produced greater inhibition than their methylglycosides, and therefore Hermentin *et al.* (1984*b*) suggest that the aminophenyl moiety used for covalent attachment of sugar to BSA could have partly explained the marked inhibition observed by Jungery *et al.* (1983*a*). However this does not explain the lack of inhibition resulting from lactose linked to BSA via a similar aromatic spacer.

Despite these possible effects of toxicity, the role of GlcNAc in attachment of merozoite to the glycophorins is further supported by Jungery *et al.* (1983*b*) who found that GlcNAc (300 mM) was specifically capable of eluting parasite proteins from glycophorin coupled to Sepharose-4B and that the immobilized sugar itself was capable of specifically binding metabolically labelled parasite molecules.

The inhibition of invasion by chitobiose [GlcNAc(β1–4)GlcNAc] and chitotriose also supports a role of the N-linked oligosaccharide (Howard *et al.* 1982; Hermentin *et al.* 1983). In implicating the N-linked oligosaccharide, Hermentin *et al.* (1984*a*) conclude that a core rather than an outer component of this oligosaccharide is involved since GlcNAc(β1–4)ManOMe (Man-mannose) and chitobiose and chitotriose rather than Gal(β1–4)GlcNAcOMe were inhibitory (see figure 1). The importance of the N-linked sugar might also explain the less potent inhibition of equivalent amounts of δ (which has no N-linked sugar) when compared to α (Perkins 1984). A precedence for the importance of both O- and N-linked sugar exists in the binding of wheatgerm agglutinin to α which is thought to involve both sugars (Bhavandanan & Katlic 1978). One further attractive aspect of implicating N-linked oligosaccharides is that they have been found to be highly species specific in a number of serum glycoproteins (Berger *et al.* 1982), and could thus explain at least in part the species specificity of some *plasmodia* for a particular type of red cell (Butcher *et al.* 1973).

The role of membrane lipid

Thus far there is no evidence to suggest that any receptor for *P. falciparum* on red cells might involve lipid. Perkins (1981) could detect no invasion inhibition by the lipid and glycolipid present in the chloroform: methanol phase of membrane protein fractionation. Polyglycosylceramides were found to have no effect on invasion (Schulman *et al.* 1983). A mixture of gangliosides from sheep brain was similarly without effect (W. V. Breuer and Z. I. Cabantchik, personal communication).

Invasion inhibition by monoclonal antibodies and lectins

Numerous monoclonal antibodies directed against various portions of the red cell sialoglycoproteins have now been prepared (Anstee & Edwards 1982; Ridgwell *et al.* 1983; Anstee *et al.* 1984*a*) and the effect of some of these on invasion has been published (Jungery 1983). In a further set of experiments the inhibition by both ascites and protein A-purified antibody has been examined (table 2).

The most marked inhibition was seen with the antibody B14 directed against the Wr^b antigen which is thought to be located on α at a site near the membrane (Ridgwell *et al.* 1983). Some inhibition at sub-agglutinating doses was also seen using R10, a monoclonal directed against the trypsin sensitive portion of α. Three other monoclonals against the Wr^b antigen (R7, B13

TABLE 2. INVASION OF RED CELLS BY *P. FALCIPARUM* IN PRESENCE OF ANTI-GLYCOPHORIN MONOCLONAL ANTIBODIES

		ascites				purified antibody				
			% control§					% control§		
antibody†	IgG isotype	reciprocal direct‡ agglutination titre	dilution¶ 10^{-1}	10^{-2}	10^{-3}	reciprocal direct agglutination titre	final neat concentration $\mu g\ ml^{-1}$	dilution¶ neat	10^{-1}	10^{-2}
R1.3	1	10^3	+ 95	86	79	10^2	80	+ O	85	112
R6A	1	n.d.	93	118	89	n.d.	n.d.	n.d.	n.d.	n.d.
R10	1	10^4	+ 0	+ 0	68	10	80	47	105	119
R18	2b	10^4	+ 9	+ 45	107	10^4	110	+ 11	70	98
B4	1	10^3	77	75	90	n.d.	8	82	100	82
B14	2a	$> 10^6$	+ 0	+ 23	38	10^4	70	+ 4	52	52

+, Indicates visible agglutination.
O, Counting made impossible by agglutination.
n.d., Not done.
† The antibody specificities are as follows: (i) R1.3 reacts with the amino-terminal end of α and δ and is neuraminidase-sensitive. (ii) R6A fails to react with rhesus null cells. (iii) R10 reacts with the trypsin-sensitive portion of α. (iv) R18 reacts with the trypsin-resistant portion of α further away from the membrane than the Wr^b determinant. (v) B4 reacts with β. (vi) B14 reacts with the Wr^b determinant on α close to the membrane (see Anstee & Edwards 1982; Ridgwell *et al.* 1983; Anstee *et al.* 1984*a*).
‡ 100 μl of 3% cell suspension added to 100 μl of antibody dilution (that is, further dilution 1:2).
§ Controls for these experiments 5.8 rings per 100 red cells.
¶ 150 μl of 1.5% infected cell suspension added to 50 μl of antibody dilution (that is, further dilution 1:4).

and B15) have all shown inhibition of parasite invasion at sub-agglutinating doses (G. Pasvol unpublished).

Lectins have also been tested to a limited extent to determine whether they are capable of inhibiting invasion. The most noticeable activity was with wheatgerm agglutinin which showed 67 and 59% inhibition at concentration of 50 and 10 $\mu g\ ml^{-1}$ respectively. Monovalent succinylated wheatgerm agglutinin showed 50 and 25% inhibition at the same concentrations whereas soybean agglutinin was without effect (Perkins 1984).

Interpretation of the data using monoclonal antibodies and lectins is difficult. The most obvious problem is that of agglutination of red cells, although it has been noted that R1.3, a strongly agglutinating antibody, does not inhibit invasion (table 2). Antibodies and lectins may furthermore inhibit invasion by a number of non-specific mechanisms such as steric hindrance and crosslinking of determinants on adjacent glycophorins. Most recently it has been shown that R10 and even its Fab′ fragment may decrease membrane deformability by possibly inducing an association between α and the red cell cytoskeleton when they bind to the cell (Chasis *et al.* 1983). Red cells preincubated with wheatgerm agglutinin also became less deformable. This was certainly not the case with all the monoclonals listed in table 2 as normal invasion was seen with many. However all these factors indicate that the data using antibodies and lectins need to be interpreted with caution.

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The role of electrostatic interactions

The progressive loss of sialic acid from the surface of red cells deficient in α, β, γ or δ is paralleled by an increasing inability of the parasite to attach to or enter such cells. This might suggest that the role of the glycophorins is mainly one of providing electrostatic interactions with merozoites, determined largely by the presence of sialic acid. Friedman (1983) has in fact proposed that this is the mechanism whereby α_1-acid glycoprotein (AGP) inhibits invasion *in vitro*, that is, by virtue of its negative charge. He found that desialylated AGP lost its ability to inhibit invasion, whereas unrelated non-protein polyanions such as heparin, dextran sulphate, inositol hexaphosphate and hexasulphate were inhibitory. Furthermore, AGP could partly reinstate the lost of invasion resulting from trypsin or neuraminidase treatment of human red cells (Friedman *et al.* 1984). Invasion appeared to parallel measured red cell charge.

There are a number of inconsistencies with the hypothesis that surface charge on glycophorin determines recognition by the merozoites. First, the effect on invasion of neuraminidase treatment which removes most of the negative charge is less than that produced by trypsin which removes substantially less sialic acid (Miller *et al.* 1977). Furthermore, Tn cells which contain about 46% of the sialic acid content of normal cells are almost totally resistant to invasion (Pasvol *et al.* 1982*b*) and even more striking is the resistance of Cad cells which resist infection with a full complement of sialic acid (Cartron *et al.* 1983). The charge hypothesis certainly does not apply to all species of plasmodia; invasion of human cells by *P. knowlesi* is unaffected by neuraminidase treatment (Miller *et al.* 1977). Finally, the reduced negative charge of merozoites of *P. falciparum* compared to rings and trophozoites (Heidrich *et al.* 1982) presumably favours the interaction of merozoite and negatively charged red cell. Any modification of the red cell leading to a reduction in negative charge alone (such as neuraminidase treatment) would be expected to facilitate rather than inhibit such an interaction. It is concluded that although charge may well modify merozoite–red cell interplay, charge alone is not consistent with the accumulated data to explain the degree of specific recognition of human red cells by merozoites of *P. falciparum*.

The role of portions of α close to the membrane and hydrophobic interactions

The glycophorins are amphiphilic molecules with well hydrated carbohydrate-rich outer amino-terminal ends, and inner hydrophobic segments associated with the membrane which are possibly the site of glycophorin dimeric association (Furthmayr & Marchesi 1976). One determinant in particular, the Wr^b antigen has been found to be located close to this inner hydrophobic portion of α (Ridgwell *et al.* 1983) and cells negative for the Wr^b antigen taken from the only known individual who has this variant as an isolated defect were found to be resistant to infection (Pasvol *et al.* 1982*b*). These findings could not be confirmed by others (P. David, M. E. Perkins and L. H. Miller, personal communication). Evidence for the participation of hydrophobic interactions in invasion has come from Breuer *et al.* (1983*a*) and Perkins (1984) who found that the T6 tryptic fragment (residues 53–92) and other smaller hydrophobic peptides of α inhibited invasion. Breuer *et al.* (1984) have also demonstrated that BSA derivatized with hydrophobic moieties such as nitrobenzylfurazan, dinitrobenzyl, trinitrobenzyl and dansyl groups inhibit invasion. Other proteins, such as human serum albumin, transferrin, ovalbumin, fetuin and casein derivatized with dinitrobenzyl groups were all inhibitory. When BSA was gradually substituted with 2,4-fluorodinitrobenzene it inhibited

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invasion in a dose-dependent manner. They concluded that hydrophobic interactions were important in the invasion process.

Hydrophobic portions of the glycophorins could aggregate reversibly, and the formation or dissociation of oligomers for example has been suggested to serve as an on–off switching mechanism for various hormones and ligands (Marchesi 1978). Some as yet unknown factor altering this on–off switch might explain the differences in the invasion rates observed in Wr^b-ve cells.

The role of other non-specific interactions

Although the initial recognition of the target red cell by the merozoite may involve only relatively few determinants, the invasion process is more than likely a highly complex interaction involving many biochemical events. Increased membrane rigidity for example, quite independent of surface sialoglycoproteins, may be a factor resulting in decreased invasion of Melanesian ovalocytic cells (Mohandas *et al.* 1984). Decreased membrane deformability may similarly account for the decreased invasion of metabolically old cells and cells containing haemoglobins S and C (Pasvol & Wilson 1982). Inhibition of the interaction between elements of the red cell cytoskeleton by anti-spectrin antibodies introduced into the cell or by cross-linking agents impeded parasite entry (Dluzewski *et al.* 1983), while incorporation of a polar cholestrol derivative such as cholestrol hemisuccinate into the membrane of normal red cells led to resistance to invasion (Breuer *et al.* 1983*b*). This was thought to be due to a decrease in the vertical and lateral mobility of membrane components rather than a decrease in membrane deformability.

3. Merozoite antigens binding to the glycophorins

Numerous *P. falciparum* merozoite antigens have now been identified and partly characterized (reviewed by Newbold 1984). However only a few of these molecules have been located on the merozoite surface where they can participate in the initial contact with the glycophorins (Perrin *et al.* 1981; Perkins 1981; Hall *et al.* 1983; Freeman & Holder 1983; Heidrich *et al.* 1983). Despite the apparent complexity of the interactions between merozoites and red cells, relatively few parasite components have been found to specifically bind to the glycophorins. Thus Jungery *et al.* (1983*b*) have identified two metabolically labelled parasite proteins which bind to Sepharose-4B-coupled glycophorins. These were of M_r 140000 and 35000 respectively and were specifically eluted with GlcNAc (300 mM). Moreover, GlcNAc when coupled to Sepharose also bound metabolically labelled parasite molecules of similar M_r (that is, 140000 and 35000) and in addition a molecule of 70000. The relationship of these three molecules (140000, 70000 and 35000) is intriguing, for they could represent tetramer, dimer and monomer, processed parts of a larger molecule, or an artefact of *in vitro* proteolysis. Perkins (1983) has identified a metabolically labelled parasite molecule of M_r 155000 which adsorbs to glycophorin. Polyclonal antibodies to this protein have shown that it is located on the surface of the merozoite and have been found to inhibit partly invasion *in vitro* (M. E. Perkins, personal communication). Numerous critical questions about these glycophorin-binding parasite polypeptides need to be answered before their relevance as immunogens can be ascertained. Nevertheless their identification has at least indicated the feasibility of isolating parasite molecules by this method.

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4. Conclusions

Unfortunately little is known of the three dimensional structures or arrangement of the glycophorins as they exist on the intact cell. The outer surface of the red cell as envisaged by our current knowledge would be that of a carbohydrate-rich negatively charged lattice bonded by localized or extended hydrogen bonds (Tanner 1978). This would provide a hydrophilic, highly hydrated barrier, and surface receptors for initial attachment of merozoites would thus have to be external to this barrier. Once attached, merozoites would have to penetrate or disrupt this network to interact with more internal components of the membrane. Any obstacle to the initial recognition event (be it immunological or pharmacological) would lead to failure of infection of red cells and thus failure of the development of disease.

The data presented in this paper are still consistent with out initial hypothesis on the mechanism of invasion (Pasvol *et al.* 1982*b*). Alterations in surface charge such as the decreased negative charge of merozoites facilitate initial contact with a susceptible cell. Lectin-like molecules on the surface coat of the merozoite specifically recognize clusters of oligosaccharides, possibly on all of the glycophorins and specified mainly by NeuNAc and GlcNAc on both the O- and N-linked sugars in a manner not unlike that of the binding of wheatgerm agglutinin to specific sialoglycoconjugates. Once attachment has occurred, interactions necessary for the process of junction formation and red cell deformation follow, and these might involve the more internal portions of the glycophorins close to the membrane (and possibly close to the Wr^b determinant). These events lead to more generalized changes which require deformability of the membrane, rearrangement of the underlying cytoskeleton and finally parasite entry.

Whatever the mechanisms of entry are, or why and how they occur, it is nevertheless reassuring that only a few parasite proteins of *P. falciparum* have thus far been found to bind to the human glycophorins. Whether these molecules will be of value as components of a malarial vaccine remains as yet unanswered.

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