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Plasmodium falciparum: A Family of Sulphated Glycoconjugates Disrupts Erythrocyte Rosettes

ALEXANDRA ROWE, ANTHONY R. BERENDT, KEVIN MARSH,* AND CHRISTOPHER I. NEWBOLD

*Molecular Parasitology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, OX3 9DU, United Kingdom; and *Kenya Medical Research Institute, Coastal Unit, P.O. Box 230, Kilifi, Kenya*

ROWE, A., BERENDT, A. R., MARSH, K., AND NEWBOLD, C. I. 1994. *Plasmodium falciparum*: A family of sulphated glycoconjugates disrupts erythrocyte rosettes. *Experimental Parasitology* 79, 506–516. The ability of *Plasmodium falciparum*-infected erythrocytes to form spontaneous rosettes with uninfected red cells is a parasite adhesion property which has been associated with severe malaria. The mechanism of rosetting remains unknown, but the ability of heparin to disrupt rosettes has been recognised previously. In this paper we show that a group of sulphated glycoconjugates including sulphatide, dextran sulphate, and fucoidan are more effective rosette reversing agents than heparin and are active against both laboratory strains and wild isolates. Other related anionic glycosaminoglycans such as the chondroitin sulphates A, B, and C and hyaluronic acid have no effect on rosette formation. This family of sulphated glycoconjugates which are active against rosettes is also known to inhibit sporozoite invasion of hepatocytes and merozoite reinvasion of erythrocytes, suggesting that sulphated glycoconjugate interaction may be an important process in cell adhesion at different stages in the plasmodial life cycle.

INDEX DESCRIPTORS AND ABBREVIATIONS: *Plasmodium falciparum;* protozoa; sulphated glycoconjugate; rosette formation; parasitised red blood cell (PRBC); uninfected red blood cell (RBC); Malayan Camp Rosetting strain (MCR); glycosaminoglycan (GAG); *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK); phosphate-buffered saline (PBS); circumsporozoite (CS); thrombospondin-related anonymous protein (TRAP); sporozoite surface protein 2 (SSP2).

INTRODUCTION

Severe *Plasmodium falciparum* malaria is thought to be linked to the adhesion properties of parasitised erythrocytes, which may cytoadhere to endothelial cells or form rosettes with uninfected erythrocytes. Rosetting has been detected in parasite isolates from around the world (Wahlgren *et al.* 1990) and is more frequent in isolates from children with cerebral malaria than mild malaria (Carlson *et al.* 1990a; Treutiger *et al.* 1992). Rosetting may contribute to the pathogenesis of severe malaria by causing obstruction to blood flow in the microvasculature (Kaul *et al.* 1991), and some post-mortem studies have noted the presence of rosette-like aggregates of uninfected and infected erythrocytes in congested cerebral vessels (Pongponratn *et al.* 1991; Riganti *et al.* 1990) and in the retina (Hidayat *et al.* 1993).

Rosetting has been described as a temperature-independent, but pH- and calcium-dependent phenomenon which is sensitive to proteases and heparin (Carlson *et al.* 1990b). Monoclonal antibodies to CD36 (Handunnetti *et al.* 1992a) and to the plasmodial antigen PfHRP1 (Carlson *et al.* 1990b) disrupt rosettes in some strains. The molecular mechanisms of rosette formation are unclear, but it has been suggested that rosetting may be mediated by lectin–carbohydrate interactions between parasite-derived strain-specific "rosettins" (Helmby *et al.* 1993) and red cell oligosaccharides (Carlson and Wahlgren 1992).

The sensitivity of rosettes to heparin has been recognised for several years (Udomsangpetch *et al.* 1989), and the ability of

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different heparin fractions to disrupt rosettes has been described (Carlson *et al.* 1992). Heparin is a highly sulphated GAG which is found in the granules of connective tissue mast cells and basophils and is related to the more commonly found heparan sulphate proteoglycans. Several pathogenic microorganisms such as *Trypanosoma cruzi* (Ortega-Barria and Pereira 1991), *Chlamydia trachomatis* (Zhang and Stephens 1992), *Herpes simplex* virus (Shieh *et al.* 1992), and cytomegalovirus (Neyts *et al.* 1992) bind to host cells via heparan sulphates, and these interactions can be inhibited by heparin.

We considered the possibility that the ability of heparin to disrupt rosettes may reflect the involvement of heparin-like sulphated glycoconjugates in rosette formation and therefore performed detailed studies of the effect of a range of sulphated glycoconjugates on rosetting. We describe the rosetting characteristics of two laboratory lines, R29 and MCR, and show that a group of sulphated glycoconjugates including dextran sulphate, fucoidan, and sulphatide are active against rosetting in both laboratory strains and wild isolates.

Materials and Methods

Reagents and Antibodies

All sulphated glycoconjugates and glycolipids were from the Sigma Chemical Co. (Poole, Dorset, UK), as were trypsin (TPCK-treated), trypsin inhibitor type III-O, and cycloheximide. Sodium fluoride and sodium azide were from BDH (Lutterworth, Leicestershire, UK), and sodium chlorate was from the Aldrich Chemical Co., Ltd. (Gillingham, Dorset, UK). *Vibrio cholera* neuraminidase and chymotrypsin were from Boehringer-Mannheim (Lewes, East Sussex, UK). MAb 89 was a gift of Dr. D. Taylor (Georgetown University, Washington DC) and Sulph 1 MAb was a gift of Dr. P. Fredman (University of Stockholm, Sweden). OKM5 and OKM8 were a gift of Dr. P. Rao (Orthopharmaceuticals, Raritan, New Jersey).

Parasites

R29 is a cloned parasite line derived from the IT strain as previously described (Roberts *et al.* 1992). MCR (Handunnetti *et al.* 1992b) was a gift of Dr. R. Howard (Affymax, California). Parasites were cultured in human group O erythrocytes using RPMI 1640 medium supplemented with 2 m*M* glutamine, 37.5 m*M* Hepes, 20 m*M* glucose, 25 μg/ml gentamicin, and 10% normal human serum and gassed with a mixture of 96% nitrogen, 3% carbon dioxide, and 1% oxygen. Parasites were selected once a week by sedimentation in gelatin to maintain the rosetting phenotype (Handunnetti *et al.* 1992b) and were synchronised once to twice a week by sorbitol lysis (Lambros and Vanderberg 1979). Wild isolates were collected from patients with malaria at Kilifi District Hospital, Kenya, and were cryopreserved in glycerolyte and stored in liquid nitrogen. Wild isolates were thawed and grown for at least 24 hr *in vitro* to the mature pigmented trophozoite stage before use.

Assessment of Rosette Frequency

An aliquot of culture suspension was stained with 20 μg/ml ethidium bromide and a wet preparation viewed under fluorescence (Zeiss Universal microscope, 40X objective). One hundred PRBC were counted and scored for the number of RBC bound, with the presence of 2 or more RBC constituting a rosette. The rosette frequency is the proportion of mature (trophozoite or schizont)-infected erythrocytes involved in rosettes, expressed as a percentage of total mature-infected erythrocytes. Triplicate aliquots were counted for every culture and condition studied, and experiments were blinded whenever possible. Rosette frequency, although stable within a given cycle, can vary considerably from cycle to cycle for unknown reasons. Studies were carried out on R29 and MCR at 5–10% parasitaemia, 1–2% haematocrit, with rosette frequencies of 40–80% for R29 and 20–60% for MCR.

Characterisation of Rosetting Lines

Temperature, pH, divalent cations, and metabolic inhibitors. Cultures were incubated for 1 hr at various temperatures and at a range of pH values (6.2 to 8.6) in complete RPMI. To determine divalent cation dependence mature cultures were incubated with 5 m*M* EDTA for 1 hr, and to determine the effect of various metabolic inhibitors, incubations were carried out for 4 hr.

Enzymes. Cells were incubated for 5 min at 37°C with 1 mg/ml trypsin followed by 2 mg/ml trypsin inhibitor or with 2000 U chymotrypsin followed by 0.1 mM TPCK. Cells were washed twice and resuspended in complete RPMI for 30 min at 37°C before rosettes were counted. Control cultures were mock treated, washed, and incubated as above. Cells were treated with 0.1 U *V. cholera* neuraminidase in RPMI at p*H* 6.0 for 2 hr and compared to controls incubated at pH 6.0 with no enzyme.

Antibodies. Antibodies were added to 20 μl aliquots of parasite culture suspension and incubated for 30

min at 37°C before assessment of rosette frequency. MAb89 (anti-PfHRP1) was used at a 1 in 5 dilution of mouse ascites, OKM5 and OKM8 (anti-CD36) were used at 50 μg/ml, and Sulph 1 (anti-sulphatide) was used at 100 μg/ml. Triplicate samples were prepared for each antibody and compared to isotype matched controls.

Rosette Reformation Experiments

In order to study the effects of enzymes on the rosetting properties of PRBC and RBC treated independently, it was necessary to purify PRBC free from uninfected red cells. This was done by a modification of the method of Handunetti *et al.* (1992b), using 200 μg/ml fucoidan to disrupt rosettes prior to gelatin flotation in the presence of 1 mg/ml fucoidan. Purified PRBC collected from the top layer were then washed four times to remove any residual fucoidan. This commonly yielded parasitaemias of 30–60%, and such PRBC when mixed with fresh red cells readily reformed rosettes to the original frequency.

To determine relative sensitivity to enzymes, PRBC and RBC were treated with trypsin or chymotrypsin as above. Enzyme-treated PRBC were then resuspended in complete RPMI with fresh untreated RBC and incubated for 2 hr at 37°C to allow rosettes to reform. Similarly, enzyme-treated RBC were mixed with untreated PRBC and rosette reformation was monitored. In all cases rosette frequencies were compared to controls in which the cells were mock enzyme-treated.

Sulphated glycoconjugates

A range of sulphated polysaccharides and GAGs in PBS were added to 100-μl aliquots of culture suspension at a concentration of 100 μg/ml and incubated for 1 hr at 37°C. Glycolipids in methanol were similarly added at 100 μg/ml and incubated for 1 hr. The volume of methanol added did not exceed 1% and did not cause lysis of red cells. Thin films were examined after incubation of parasites with sulphated glycoconjugates and no morphological changes were detected. The dose-dependent effects of heparin, fucoidan, dextran sulphate, chondroitin sulphates A, B, and C, and hyaluronic acid were tested over a range of concentrations from 0.1 μg/ml to 1 mg/ml. Duplicate samples at each concentration were compared to controls with PBS as additive, to determine the rosette frequency as a percentage of the control value. The effect of sulphatide on rosetting was determined over a range of concentrations from 10 to 100 μg/ml and the effect of suramin on rosetting was tested over a range of concentrations from 1 μg/ml to 1 mg/ml. The effect of sulphated glycoconjugates on wild isolates was tested at 200 μg/ml.

RESULTS

Characterisation of rosetting lines. Rosetting in R29 and MCR was found to be independent of temperature, pH, divalent cations, and metabolic inhibitors (Fig. 1 and Table I). Treatment of parasite cultures with neuraminidase had no effect on rosetting, but trypsin and chymotrypsin both abolished rosetting completely (Table I). R29 rosettes were partially reversed by a 1 in 5 dilution of MAb89 (anti-PfHRP1) but MCR rosettes were not affected, whereas the anti-CD36 monoclonals OKM5 and OKM8 partially reversed MCR rosettes but had no effect on R29 (Table I).

Rosette reformation after protease treatment. To determine whether the protease

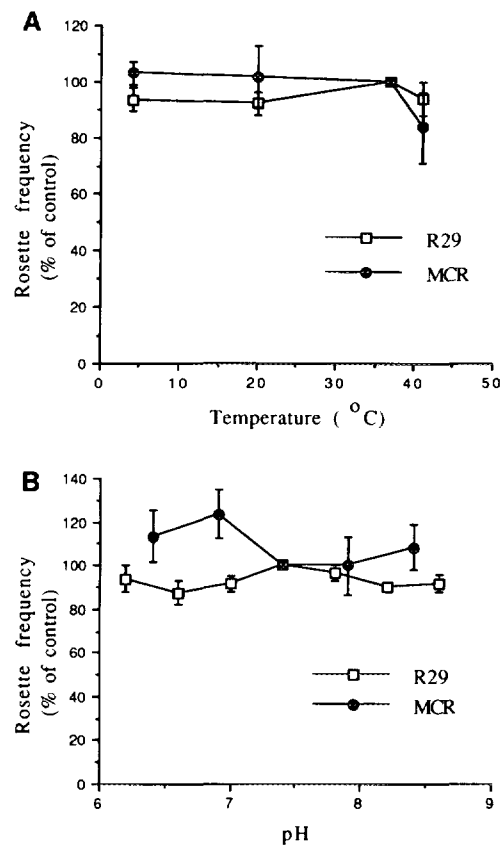

FIG. 1. Effect of temperature and pH on *P. falciparum* rosetting. (A) Cultures were incubated at various temperatures for 1 hr and compared to a control culture incubated at 37°C. (B) Cultures were incubated at various pHs in RPMI for 1 hr at 37°C and compared to a control culture incubated at pH 7.4. Graphs show mean and standard error from three experiments.

TABLE I
Rosetting Characteristics of Two *P. falciparum* Laboratory Strains

	R29	MCR
5 m*M* EDTA[a]	91 (7)	111 (11)
Metabolic inhibitors[b]		
2 m*M* Sodium fluoride	102 (3)	100 (15)
2 m*M* Sodium azide	104 (2)	117 (4)
10 m*M* Sodium chlorate	102 (7)	98 (13)
0.1 m*M* Cycloheximide	98 (5)	114 (8)
Enzymes[b]		
Neuraminidase	107 (9)	86 (5)
Trypsin	0 (0)	0 (0)
Chymotrypsin	0 (0)	0 (0)
Antibodies[a]		
MAb89 (1/5 dilution)	31 (10)	108 (4)
OKM5 (50 μg/ml)	90 (3)	37 (14)
OKM8 (50 μg/ml)	89 (1)	54 (13)

[a] Mean rosette frequency as % of control value (standard error) of three independent experiments.

[b] Mean rosette frequency as % of control value (standard error) of triplicate determinations from a representative experiment.

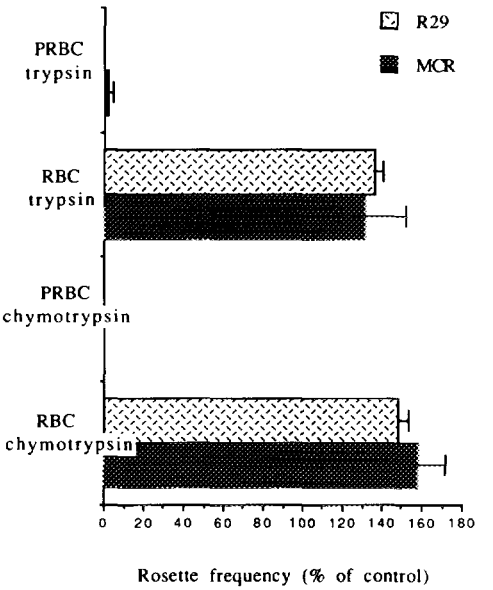

FIG. 2. Effect of proteases on *P. falciparum* rosette reformation. Purified PRBC or RBC were treated with enzymes as described under Materials and Methods, and the ability of these cells to reform rosettes was compared to control mock-treated cells. Graphs show mean and standard error from three experiments.

sensitive molecules were on the PRBC or RBC surface, purified PRBC or fresh RBC were enzyme treated separately and then mixed with untreated RBC/PRBC, and the reformation of rosettes was assessed. Trypsin- or chymotrypsin-treated PRBC were unable to reform rosettes (Fig. 2), suggesting that the parasitised cell rosetting ligand(s) is borne on proteins or glycoproteins. Treatment of RBC on the other hand enhanced rosetting (Fig. 2), suggesting that the red cell rosetting receptor(s) could be located on carbohydrates, lipids, or protease-resistant glycoproteins.

Effect of sulphated glycoconjugates. Previous studies have shown that heparin disrupts rosettes in some, but not all rosetting laboratory strains and wild isolates (Carlson *et al.* 1992). We examined the effect on rosetting of heparin and a range of related GAGs and sulphated polysaccharides. R29 and MCR showed similar patterns of rosette reversal, with fucoidan and high-molecular-weight dextran sulphate (average molecular weight 500,000) being the most effective compounds, closely followed by heparin (Fig. 3). Size and charge seem to play some part in the action of these compounds, as low-molecular-weight dextran sulphate (average molecular weight 5000) and de-*N*-sulphated heparin were less effective rosette disruptors. However, there is a degree of specificity involved as other related highly sulphated compounds such as the chondroitin sulphates A, B, and C, and the anionic unsulphated GAG hyaluronic acid, had no effect on rosetting even at concentrations up to 1 mg/ml (Fig. 4). Higher concentrations of these compounds resulted in aggregation of red cells and rouleaux formation, and so made assessment of rosette frequency impossible. Examination of 10 wild isolates showed a similar pattern of susceptibility with >50% rosette disruption occurring in all cases with fucoidan, dextran sulphate, or heparin at 200 μg/ml, with the most effective com-

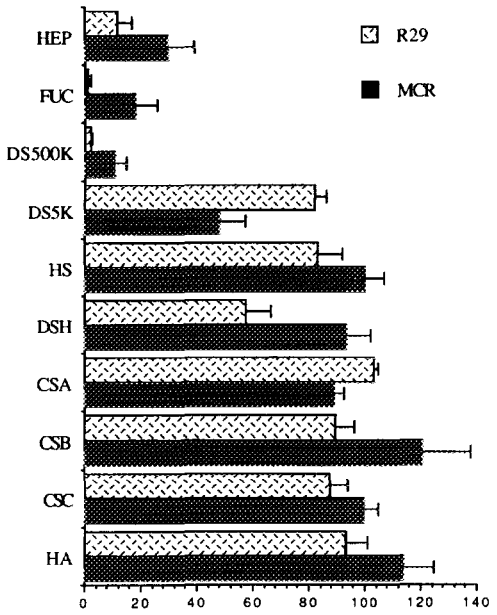

FIG. 3. Effect of sulphated glycoconjugates on *P. falciparum* rosetting. Cultures were incubated for 1 hr at 37°C with 100 μg/ml of sulphated glycoconjugate and compared to control cultures without added sulphated glycoconjugate. Graphs show mean and standard error from four experiments. HEP (heparin); FUC (fucoidan); DS500K (dextran sulphate average molecular weight 500,000); DS5K (dextran sulphate average molecular weight 5000); HS (heparan sulphate); DSH (de-*N*-sulphated heparin); CSA (chondroitin sulphate A); CSB (chondroitin sulphate B); CSC (chondroitin sulphate C); HA (hyaluronic acid).

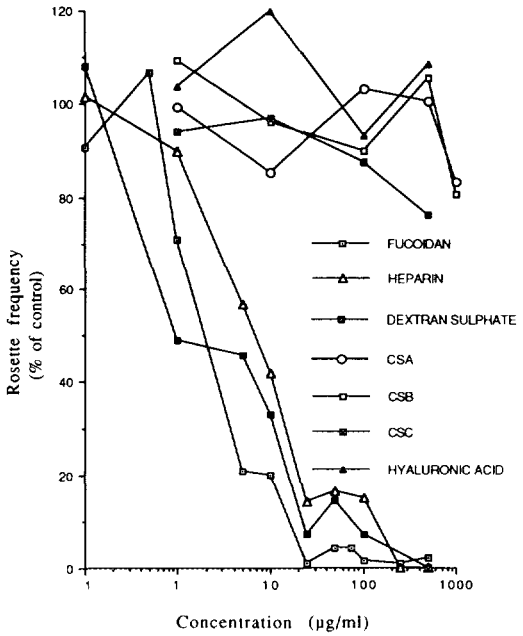

FIG. 4. Dose-dependent effects of sulphated glycoconjugates on *P. falciparum* rosetting. R29 cultures were incubated with sulphated glycoconjugates for 1 hr at 37°C and compared to control cultures with no added sulphated glycoconjugate. Graphs show the mean of duplicate determinations of rosette frequency from a representative experiment. CSA (chondroitin sulphate A); CSB (chondroitin sulphate B); CSC (chondroitin sulphate C).

pound varying from one isolate to another (Table II).

Sensitivity to fucoidan, dextran sulphate, and heparin is characteristic of interactions among many proteins such as thrombospondin, laminin, and von Willebrand factor which bind to the sulphated glycosphingolipid sulphatide (galactose-3-SO_4-β-1-ceramide) (Roberts *et al.* 1986a). We therefore looked at the effect of sulphatide on rosetting. We found that sulphatide, but not its sulphate-free homologue galactosylceramide, reversed rosettes in R29, MCR, and the 10 wild isolates (Fig. 5A), in a dose-dependent fashion (Fig. 5B). None of the other lipids studied including lactosylceramide, phosphatidylcholine, phosphatidylinositol, phophatidylethanolamine, gangliosides type II, sphingomyelin, cholesterol, and cholesterol-3-SO_4 had any significant effect on rosetting (data not shown). Sulphatide is a component of normal red cell membranes (Hansson *et al.* 1978), so we therefore considered the possibility that sulphatide may play a direct role in the mechanism of rosette formation. A monoclonal antibody to sulphatide, Sulph1 (Fredman *et al.* 1988) was added to R29 and MCR at a concentration of 100 μg/ml, but it failed to reverse rosettes or prevent rosette reformation (data not shown). Using Sulph1 we could not detect sulphatide on the erythrocyte membrane by flow cytom-

TABLE II
Effect of Sulphated Glycoconjugates on *P. falciparum* Wild Isolates

Isolate	Rosette frequency[a]				
	Heparin	Fucoidan	DEX S[b]	CSA[c]	HA[d]
K1	63 (11)	30 (13)	82 (27)	108 (33)	121 (15)
K2	54 (17)	28 (10)	3 (1)	100 (6)	96 (14)
K3	0 (0)	0 (0)	1 (1)	71 (6)	84 (2)
K4	0 (0)	13 (2)	2 (2)	110 (39)	56 (11)
K5	19 (17)	0 (0)	2 (1)	54 (4)	96 (4)
K6	5 (3)	8 (8)	15 (10)	95 (9)	79 (26)
K7	1 (1)	1 (1)	0 (0)	65 (1)	104 (18)
K8	65 (12)	0 (0)	0 (0)	87 (5)	96 (9)
K9	99 (7)	59 (4)	0 (0)	117 (3)	107 (2)
K10	54 (7)	4 (2)	5 (4)	113 (26)	119 (15)

[a] Percentage of control value (standard error) of triplicate determinations from a single experiment. Isolates were incubated for 1 hr at 37°C with 200 μg/ml of sulphated glycoconjugate and compared to control cultures with no added sulphated glycoconjugate.
[b] Dextran sulphate, (average molecular weight 500,000).
[c] Chondroitin sulphate A.
[d] Hyaluronic acid.

etry, and this may reflect masking of sulphatide by the erythrocyte glycocalyx. Functional activity of Sulph1 was shown by its ability to prevent the disruptive effect on rosetting of added sulphatide (data not shown). It remains possible that sulphatide or more complex red cell sulphated glycolipids could be involved in rosette formation.

Finally we tested the effect of the antiprotozoal drug suramin on rosetting. Suramin is a polysulphonated drug which has been used in the treatment of African trypanosomiasis, onchocerciasis, cancer, and AIDS (Voogd *et al.* 1993) and is known to inhibit the interaction of several adhesive proteins with sulphatide (Zabrenetzky *et al.* 1990) and the invasion of HepG2 cells by *P. berghei* sporozoites (Muller *et al.* 1993). We found that suramin inhibited R29 rosetting in a dose-dependent manner with 50% disruption of 50 μg/ml (data not shown).

Discussion

We have shown that a group of sulphated glycoconjugates including sulphatide, dextran sulphate, and fucoidan, in addition to heparin (Udomsangpetch *et al.* 1989), disrupt *P. falciparum*-erythrocyte rosettes. The effect of these compounds is specific and not simply related to charge, as other related anionic compounds such as the chondroitin sulphates A, B, and C and hyaluronic acid have no effect on rosetting. Nash *et al.* (1992) found that the nonsulphated glucose polymer dextran 500 caused a slight decrease in rosette frequency in the Palo Alto rosetting strain; however, this was at a concentration approximately 1000-fold higher than the concentrations of sulphated glycoconjugates shown to be active in this paper. The sulphated glycoconjugates which disrupt rosettes differ in their carbohydrate constituents and overall degree of sulphation, but share the presence of a SO_4 group at the 3-carbon position in some residues. It has been suggested that the spatial orientation of sulphate esters rather than the polysaccharide backbone is important in determining the specificity of action of these compounds (DeAngelis and Glabe 1987).

Field isolates are known to differ in their

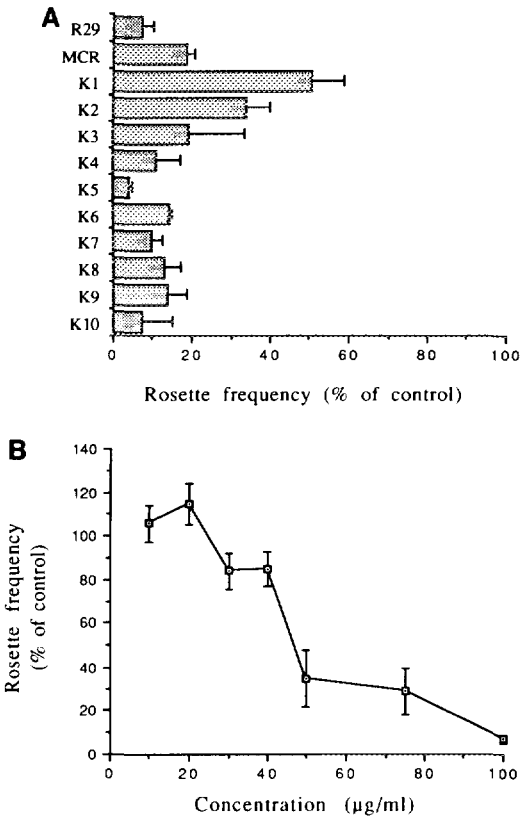

FIG. 5. Effect of sulphatide on *P. falciparum* rosetting. (A) R29, MCR, and 10 wild isolates were incubated with 100 μg/ml sulphatide for 1 hr at 37°C and compared to control cultures incubated with 100 μg/ml of the nonsulphated homologue galactosylceramide. Graphs show mean and standard error of four experiments for R29 and MCR and mean of triplicate determinations in a single experiment for the wild isolates. (B) Dose-dependent effects of sulphatide on R29 rosetting. Cultures were incubated at 37°C for 1 hr and compared to controls incubated with galactosylceramide. Graphs show mean and standard error from three experiments.

susceptibilities to rosette disruption by heparin (Carlson *et al.* 1992), so we tested a range of sulphated glycoconjugates on 10 wild rosetting isolates. We found that heparin caused >50% rosette disruption in half the isolates studied, whereas dextran sulphate, fucoidan, and sulphatide were effective against a wider range of isolates, causing >50% rosette disruption in 9 of 10 isolates in each case. All of the isolates studied were susceptible to rosette disruption by at least one of the sulphated glycoconjugates, with the most effective compound varying from one isolate to another. The ability of sulphated glycoconjugates to disrupt rosettes would therefore seem to overcome any possible heterogeneities in the mechanism of rosette formation which may exist.

This is also demonstrated by the response of the two laboratory strains MCR and R29, which show similar susceptibility to sulphated glycoconjugates despite probable differences in rosetting mechanism. MCR rosetting is inhibited by monoclonal antibodies to CD36, and it has been suggested that CD36 or a CD36-like molecule on uninfected red cells may act as a rosetting receptor for MCR (Handunetti *et al.* 1992a). R29 rosetting on the other hand is not inhibited by anti-CD36 monoclonals, but is inhibited by a monoclonal to PfHRP1 which has no effect on MCR, suggesting that R29 rosetting is mediated via a different mechanism. However, both R29 and MCR are inhibited by heparin, fucoidan, dextran sulphate, and sulphatide and not by the other sulphated glycoconjugates tested, although there are differences in the order of sensitivity to these compounds, with fucoidan being the most effective for R29 and dextran sulphate the most effective for MCR.

Sulphated glycoconjugates have been implicated in a wide variety of cell adhesion phenomena including lymphocyte trafficking (Imai *et al.* 1991), T cell-erythrocyte rosetting (Parish *et al.* 1988), neuronal cell adhesion (Cole *et al.* 1986), sperm–egg interaction (Jones and Williams 1990), and sponge cell aggregation (Parish *et al.* 1991). A diverse group of proteins have been identified, including thrombospondin (Roberts *et al.* 1985), properdin (Holt *et al.* 1990), antistasin (Holt *et al.* 1989), and von Willebrand factor (Roberts *et al.* 1986b), which bind to sulphatide and other sulphated glycoconjugates and which share the consensus protein sequence CSVTCG, which is

thought to be a sulphated glycoconjugate-binding motif (Holt *et al.* 1989). Two malarial proteins, CS protein (Pancake *et al.* 1992) and TRAP (Muller *et al.* 1993) also contain this sequence and have been shown to bind directly to sulphatide.

CS protein is present on the surface of sporozoites and is thought to be involved in hepatocyte invasion. The binding of recombinant CS protein to hepatocytes is inhibited by fucoidan, heparin, and dextran sulphate, but not by chondroitin sulphates, and it has been suggested that the hepatocyte receptor for CS protein could be a sulphated glycoconjugate (Pancake *et al.* 1992). Recently a high-molecular-weight heparan sulphate proteoglycan from hepatocytes has been identified which may fulfill this role (Frevert *et al.* 1993).

The exact function of TRAP (Robson *et al.* 1988) and its *P. yoelii* homologue SSP2 (Rogers *et al.* 1992) remains to be clarified. TRAP is thought to be expressed by sporozoites (Cowan *et al.* 1992) and by asexual erythrocytic stage parasites (Robson *et al.* 1988). If present on the infected red cell surface it could be involved in a process such as rosetting, but we have been unable to demonstrate any effect on rosetting of both monoclonal and polyclonal anti-TRAP antibodies (unpublished observations).

Merozoite invasion of erythrocytes is also inhibited by a similar group of sulphated glycoconjugates to those which inhibit rosetting and sporozoite invasion; heparin (Kulane *et al.* 1992), dextran sulphate, and fucoidan (Butcher *et al.* 1988). In *P. knowlesi* a 135-kDa fucoidan binding protein has been identified which is thought to be involved in invasion (Dalton *et al.* 1991). It remains to be seen whether parasite sulphated glycoconjugate binding proteins similar to those involved in sporozoite and merozoite invasion will turn out to be important in rosetting, but it is of interest that the same group of compounds affect cell adhesion processes at different stages in the plasmodial life cycle.

Another cell adhesion system involving sulphated glycoconjugates is lymphocyte trafficking mediated by the selectin family of cell adhesion molecules. The binding of lymphocytes to lymph node high endothelial venules is inhibited by a specific group of carbohydrates including fucoidan and mannose-6-phosphate (Stoolman *et al.* 1984), and lymphocyte L-selectin has been shown to have lectin activity for several sulphated and phosphyorylated sugars (Imai *et al.* 1990). It was presumed that these sugars represented structural mimics of the actual L-selectin ligand on high endothelial venules, and this has recently been identified as sulphated, fucosylated carbohydrate presented by a mucin-like molecule, GLYCAM1 (Lasky *et al.* 1992).

We suggest that the ability of sulphated glycoconjugates to disrupt rosettes may indicate that these compounds act as analogues of the actual ligand(s) involved in rosette formation, involving a proteoglycan-type molecule as in the case of CS protein-hepatocyte adhesion, or sulphated carbohydrate presented on glycolipids, or glycoprotein as in the case of L-selectin–GLYCAM1.

Carlson and Wahlgren (1992) have suggested that rosetting may be mediated by parasite strain-specific lectins binding to blood group oligosaccharides. The enhanced rosetting capacity to trypsin- or chymotrypsin-treated RBC which we have demonstrated in this paper is consistent with this hypothesis, although we suggest that sulphated carbohydrates could be the important red cell molecules. Alternatively, rosetting could be mediated by parasite-derived sulphated glycoconjugates binding to red cell-sulphated glycoconjugate receptors. We are currently undertaking further experiments to explore these two possibilities.

As rosetting has been associated with the development of severe malaria (Carlson *et al.* 1990a; Treutiger *et al.* 1992), it has been suggested that heparin may be useful ther-

apeutically due to its anti-rosette action (Carlson *et al.* 1992). Heparin has been used in the past as a treatment for malaria on the grounds that disseminated intravascular coagulation may be an important complication, but its usefulness is unproven and anti-coagulant side-effects can be a significant problem (Warrell *et al.* 1990). Heparin fractions with low anticoagulant activity retain the ability to inhibit some rosetting strains (Carlson *et al.* 1992), but the problem remains that some parasite isolates are not susceptible to heparin. Other compounds such as dextran sulphate or suramin could be useful for anti-rosette therapy but may also cause serious toxicity. The identification of a family of sulphated glycoconjugates with anti-rosette activity against a range of isolates suggests that it may be possible to develop small oligosaccharide analogues specific for the rosetting receptor-ligand interaction which could inhibit rosetting without causing significant side effects.

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