The effects of chemical modification on the adhesion of *Plasmodium falciparum*-infected and uninfected erythrocytes

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SUMMARY

Binding of *Plasmodium falciparum*-infected erythrocytes (PE) to endothelial cells is mediated by the erythrocyte-membrane protein, band 3-related adhesin. To determine its role, the binding of infected cells treated with various chemical modifiers was investigated. Binding was inhibited by a lysine modifier (4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS)) known to specifically bind to band 3, another lysine modifier (trinitrobenzene sulfonic acid), a tyrosine modifier (sodium iodide in conjunction with lactoperoxidase, hydrogen peroxide) and oxidants (diamide, sodium periodate and ADP-chelated ferric ion), but binding was unaffected by the histidine modifier (diethylpyrocarbonate) and the arginine modifier (phenyl glyoxyl monohydrate). To artificially expose the band 3-related adhesin, uninfected erythrocytes were treated with acridine orange or loaded with calcium. These cells bound to C32 amelanotic melanoma cells, were immunostained with a monoclonal antibody that specifically binds to the band 3-related adhesin on PE, and the binding was inhibited by this monoclonal antibody. The binding of acridine orange-treated and calcium-loaded uninfected erythrocytes, could also be blocked by DIDS. In the case of acridine orange-treated erythrocytes, the patterns of the effects of the chemical modification on binding were consistent with that of PE except for tyrosine modification. These results demonstrate that the band 3-related adhesin, even in the absence of parasite-encoded proteins, contributes to PE adhesion.

Key words: chemical modification, adhesion, *Plasmodium falciparum*, erythrocyte, malaria.

INTRODUCTION

Sequestration, the adherence of infected erythrocytes to the endothelial cells lining the capillaries and post-capillary venules of organs such as the heart, lungs, liver and brain, is characteristic of *Plasmodium falciparum* (Aikawa *et al*. 1990; Pongponratin *et al*. 1991) and often causes microvascular occlusion and, in a minority of cases, this blockage may lead to the acute pathology of cerebral malaria (MacPherson *et al*. 1985; Warrell, 1987; Howard & Gilladoga, 1989).

Approximately 16 h after parasite invasion, 'knobs' appear on the erythrocyte surface (Trager, Rudzinska & Bradbury, 1966; Gruenberg, Allred & Sherman, 1983) and these are correlated with alterations in the antigenicity of the infected cell (Howard, 1987). These novel antigens have been suggested to mediate the adherence of the parasitized erythrocyte to the endothelium and several have been described in molecular terms. A single molecule, *P. falciparum* erythrocyte membrane protein 1 (PfEMP 1), encoded by 1 of 50–100 copies of the *var* gene complex has been reported to bind to thrombospondin, CD36, intercellular adhesion molecule-1 and thrombomodulin on the luminal surface of the endothelial cell (Baruch *et al*. 1996; Newbold *et al*. 1997) whereas others claim that another parasite-encoded antigen, sequestrin, mediates adherence to CD36 (Ockenhouse *et al*. 1991). Other work suggests that in addition to these parasite proteins there may be modifications in the erythrocyte membrane protein, band 3, that can result in erythrocyte binding (Crandall *et al*. 1993; Crandall & Sherman, 1994*a*). At present, it is not known what the relative contributions each of these molecules makes to cytoadherence, the *in vitro* equivalent of sequestration.

Chemical modification of the side-chains of residues in protein antigens has been used for epitope identification. The advantage of chemically altering an antigen is that it can be applied to discontinuous as well as continuous epitopes (Young & Oomen, 1996). Chemical modifications can rarely identify a particular residue type but altering the structure of a surface determinant can change antibody binding and can also lead to a loss in function i.e. ablation of adherence. The objectives of the present work were to compare the effects of several chemical agents on the binding properties of 3 isolates of *P. falciparum* and to evaluate whether it is possible to chemically modify uninfected erythrocytes so that they mimic the properties of the PE.

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Table 1. Effect of chemical modifications on cytoadherence of PE (A), AO–E and Ca–E (B)

(PE, AO–E and Ca–E were treated with the chemical modifiers as described in the Materials and Methods section and analysed for cytoadherence. Control values of binding of FCR-3, B8B6, CS2, AO–E, and Ca–E are 2·58–11·34, 3·80–10·17, 15·30–22·05, 3·06–3·12, and 1·04 cells/melanoma cell, respectively. Representative results of 2–4 independent assays are indicated.)

	Concentration (mM)	A Inhibition of *P. falciparum*-infected erythrocyte binding			B Inhibition of uninfected erythrocyte binding	
		FCR3	CS2	B8B6	AO–E	Ca–E
DIDS	0·05	++*	++	++	+	++
NaI/LPO/H_2O_2		++	++	+	−	−
TNBS	0·2	+	+	+	++	−
DEPC	1·0	−	−	−	−	+
PG	5·0	−	−	−	−	−
Diamide	5·0	++	++	++	+	+
Sodium periodate	0·5	++	++	+	±	++
ADP/Fe^{3+}	3·4/0·2	++	+	++	+	−

* ++, > 75% inhibition; +, 50–75% inhibition; ±, 25–50% inhibition; −, < 25% inhibition.

MATERIALS AND METHODS

Materials

4,4′-Diisothiocyanostilbene-2,2′-disulfonate (DIDS) was obtained from Calbiochem (La Jolla, CA). Sodium iodide and hydrogen peroxide (30% solution) were obtained from Fisher Scientific (Tustin, CA). Phenyl glyoxyl monohydrate (PG) and ferric chloride were obtained from Aldrich Chemical Company (Milwaukee, WI) and Mallinkrodt Chemical (Paris, KY), respectively. Calcium chloride was obtained from Matheson Coleman & Bell (Norwood, OH). Other chemicals were obtained from Sigma Chemical Company (St Louis, CA).

Parasites

All *P. falciparum* lines (FCR-3 from Gambia; B8B6 (Biggs *et al*. 1990) derived from the ItG2 isolate from Brazil; and CS2 (Cooke *et al*. 1996) derived from the Brazilian Ituxi isolate) were cultured in O+ human erythrocytes as described previously (Trager & Jansen, 1976). FCR-3 is a knobby CD36-preferring line that has both PfEMP 1 and modified band 3 as adhesins (Baruch *et al*. 1996; Crandall *et al*. 1993; Crandall & Sherman, 1994*a*); B8B6 is a knobless CD36-preferring line that has PfEMP 1 and modified band 3 as adhesins, but presumably the organization of these adhesins differs from that of FCR-3 (Biggs *et al*. 1990; S. Eda unpublished observations); CS2 is a knobby line that contains a trypsin-resistant form of PfEMP 1, and binds to chondroitin sulfate (Chaiyaroj *et al*. 1994; Cooke *et al*. 1996). Cultures of 'knobby lines' (FCR-3 and CS2) were synchronized at the mature stage by gelatin flotation (Pasvol *et al*. 1978). In the case of the knobless line, B8B6, cultures were synchronized at the ring stage by sorbitol lysis of mature forms (Lambros & Vanderberg, 1980).

Treatment of uninfected erythrocytes

Acridine orange (*AO*). Blood obtained from healthy donors was washed 3 times with 5 mM sodium phosphate buffer (pH 7·4)–0·15 M NaCl (PBS) and the buffy coat was removed by aspiration. A 10% (v/v) suspension of the washed erythrocytes was treated at room temperature for 2 h with the indicated concentrations of acridine orange dissolved in PBS. Treatment with AO did not result in lysis of the erythrocytes.

Calcium loading. The washed cells were resuspended in 5 mM Tris–HCl (pH 6·8)–0·1 M KCl–0·06 M NaCl at 10% (v/v) haematocrit and incubated at 37 °C for 3 h in the presence of the indicated concentrations of $CaCl_2$ and 2 μM of the ionophore A23187. The cells were washed twice with PBS before use. Treatment with calcium resulted in < 20% lysis of the erythrocytes.

Scanning electron microscopy

For scanning electron microscopy, AO-treated erythrocytes (AO–E), and calcium-loaded erythrocytes (Ca–E) were washed once with PBS and fixed with 0·2% glutaraldehyde in PBS at room temperature for 15 min. After several washes in PBS, dehydration of the cell pellets was performed by subsequent incubation in 30, 50, 70, 90, 100% ethanol, and absolute acetone. The dehydrated cells were dried with a Balzer's union CPD20 using CO_2 as a transition fluid, followed by mounting on SEM stubs using conductive carbon tape. Mounted samples were then coated with a gold/palladium mixture

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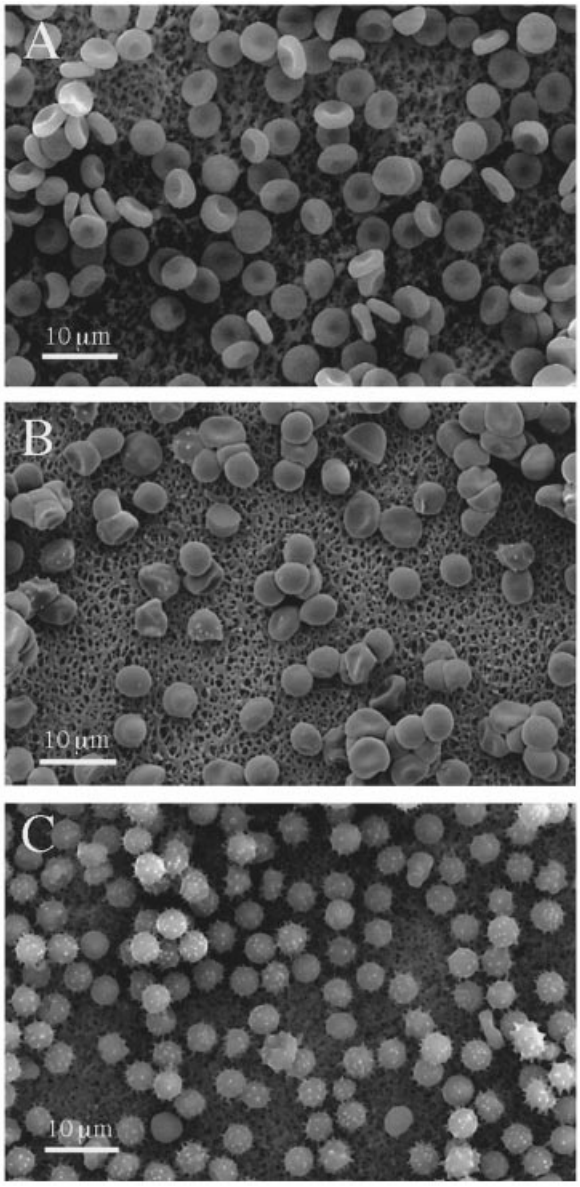

Fig. 1. Scanning electron micrographs of untreated (A), AO-treated (B) and Ca-loaded erythrocytes (C) at the magnification of ×1500.

using an EMScope SC-500 sputter coater and observed on a Phillips XL 30-FEG operated at 15 keV accelerating voltage.

Chemical modifications of erythrocytes

Suspensions of infected and uninfected erythrocytes (10% haematocrit) were treated with DIDS, diethyl pyrocarbonate (DEPC), PG, 2,4,6-trinitrobenzene sulfonic acid (TNBS), diamide, sodium periodate, and ADP-chelated ferric on (ADP/Fe^{3+}) at 37 °C for 30 min followed by washing 3 times with PBS. The cells were then tested for binding using the cytoadherence assay described below.

Iodination of erythrocyte membrane proteins was carried out: 20 μl of 5 mM sodium iodide, 100 μl of 2 mg/ml lactoperoxidase (EC 1.11.1.7) (from bovine milk, 107 unit/mg solid), and 24 μl of 0·03% hydrogen peroxide in PBS were sequentially added to 2 ml of a suspension of erythrocytes (5% haematocrit). After reaction (1 min at room temperature), 12 μl of 0·03% hydrogen peroxide was added to the suspension 3 times at 1 min intervals. The reaction was stopped by the addition of 5 ml of 5 mM NaI in PBS.

Cytoadherence

Cytoadherence assays were performed as described previously (Udeinya *et al*. 1981; Crandall, Smith & Sherman, 1991). In the case of AO–E, buffers containing acridine orange at the same concentration used for the treatment of erythrocytes, and containing 0·1% BSA, were used in the assay.

Immunofluorescent staining

Immunofluorescent staining was carried out as described previously (Crandall & Sherman, 1991).

RESULTS

To examine whether PE binding to the EC is mediated by common or different adhesins, we investigated the effects of chemical modification on the binding of 3 kinds of PE (FCR-3, B8B6 and CS2) which differentially express 1 of the well-characterized parasite-encoded adhesins, PfEMP 1, on their surface (see Materials and Methods section). As shown in Table 1A, some amino acid modifiers (DIDS for lysine residues on band 3 protein, sodium iodide in conjunction with lactoperoxidase and hydrogen peroxide (NaI/LPO/H_2O_2) for tyrosine, TNBS for lysine and tyrosine) inhibited the binding of the 3 kinds of PE (FCR-3, B8B6 and CS2) to C32 cells. All oxidants (diamide, sodium periodate, and ADP/Fe^{3+}) also inhibited the binding of the different isolates, whereas treatment with other amino acid modifiers (DEPC for histidine and PG for arginine) were without effect on PE binding. These results suggest that a common antigenic motif plays a role in PE binding; however, this binding motif appears to differ only slightly in its contribution among the different isolates. This being so it appeared that a significant portion of the binding of parasitized erythrocytes was due to an adhesin other than PfEMP 1, presumably the modified band 3 adhesin.

To confirm this suspicion, we attempted to simulate the binding of PE using uninfected erythrocytes since if these 'artificial' PE were to bind to target cells, such as C32 amelanotic melanoma cells, there would not be the involvement of a parasite-encoded adhesin. We hypothesized that aggregation and/or proteolysis of band 3 would expose the band 3-related adhesin on the PE surface, since modifications of band 3 protein have been shown to exist

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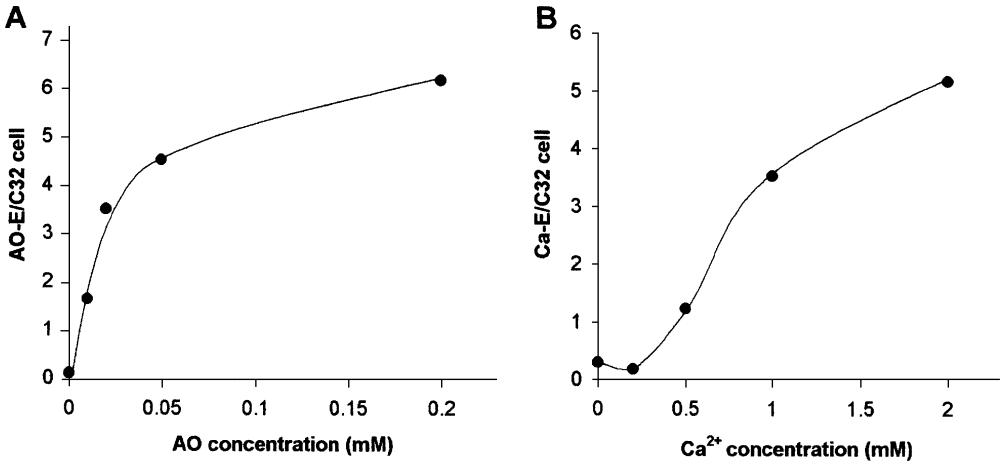

Fig. 2. Binding of AO–E (A) and Ca–E (B) to C32 melanoma cells as a function of AO and Ca^{2+} concentration. Erythrocytes were treated with various concentration of AO at room temperature for 2 h or various concentrations of Ca^{2+} in the presence of 2 μM A23187. The treated cells were analysed for their cytoadherent properties. Representative results of 2 independent assays are indicated.

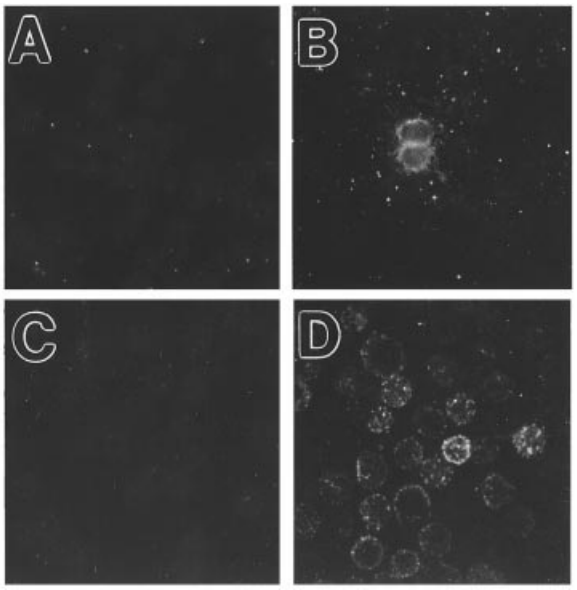

Fig. 3. Immunofluorescent staining of AO–E and Ca–E with monoclonal antibody, 3H3, produced against band 3-related adhesin. Erythrocytes were treated with or without 0·05 mM of AO at room temperature for 2 h (A, B) or 2 mM Ca^{2+} in the presence of 2 μM A23187 (C, D). The treated cells were fixed with methanol and sequentially reacted with (B, D) or without (A, C) 3H3 antibody, biotin-conjugated anti-mouse IgG antibody and fluorescein isothiocyanate-conjugated streptavidin. Photographs of the stained cells were taken using fluorescence microscopy.

on the PE membrane (Winograd & Sherman, 1989*a*, *b*; Crandall & Sherman, 1991).

Treatment of uninfected erythrocytes with AO, an agent known to cause membrane protein aggregation (Lelkes *et al*. 1983; Low & Waugh, 1985) by binding to spectrin and anionic phospholipids on the inner erythrocyte surface, resulted in a change in cell shape i.e. echinocytic and shrinking of the cells (Fig. 1B) as observed under scanning electron microscopy, and also resulted in erythrocyte binding to C32 cells. The degree of binding was directly related to the concentration of AO (Fig. 2A). Further, calcium loading of uninfected erythrocytes, which is known to cause proteolysis of band 3 in addition to membrane protein aggregation (Lorand, Siefring & Lowe-Krentz, 1978; Lorand *et al*. 1983; Murthy *et al*. 1994), also resulted in binding (Fig. 2B). In this instance, almost all of the cells showed echinocytic morphology when the cells were observed by scanning electron microscopy (Fig. 1C). When cells were treated with 0·05–0·2 mM of AO or loaded with 1–2 mM Ca^{2+}, the number of erythrocytes that bound to C32 cells was very similar to that of PE infected with the isolates FCR-3, B8B6, and CS2. Based on this finding, 0·05 mM AO and 2 mM Ca^{2+} were used in all subsequent experiments.

To determine whether the band 3-related adhesin was expressed on AO–E and Ca–E, the cells were subjected to immunostaining with the monoclonal antibody, 3H3, which specifically recognizes the band 3-related adhesin on PE. As shown in Fig. 3, AO–E and Ca–E were immunostained with 3H3 whereas no staining was observed on cells stained in the absence of 3H3 or with an irrelevant monoclonal antibody. This result indicates that AO treatment and calcium loading of erythrocytes exposes the band 3-related adhesin on the cell surface. The fact that Ca–E were not stained with 3H3 MAb and did not bind to C32 cells when calcium or A23187 were omitted during calcium loading, and that only a minority ($\sim$0·1%) of AO–E were stained with 3H3 MAb, whereas almost all of AO–E which bound to C32 cells were stained with the MAb (data not shown), demonstrates that exposure of this antigen (i.e. band 3-related adhesin) on AO–E and Ca–E is correlated with their adherence to C32 cells. Furthermore, this monoclonal antibody inhibited the binding of AO–E (Fig. 4A) and Ca–E (Fig. 4B) in a

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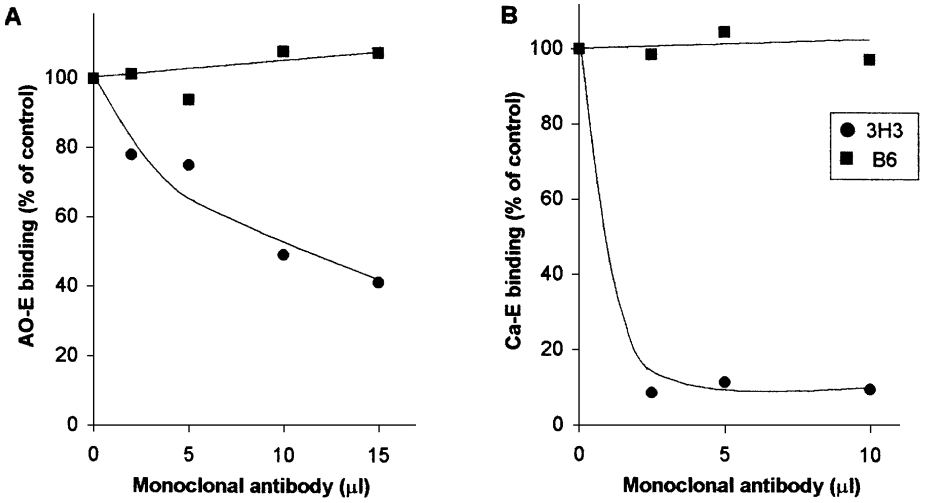

Fig. 4. Effect of monoclonal antibodies on AO–E (A) and Ca–E (B) binding to C32 melanoma cells. Erythrocytes were treated with 0·05 mM AO at room temperature for 2 h or 2 mM Ca^{2+} in the presence of 2 μM A23187. The treated cells were assayed for cytoadherence in the presence of various concentrations of 3H3 and B6 antibodies. Representative results of 2 independent assays are shown.

dose-dependent manner with a maximum inhibition of 60% and 90%, respectively. The anti-band 3 monoclonal antibody, B6, which has been shown not to inhibit PE binding, did not inhibit the binding of AO–E and Ca–E. These results, obtained using monoclonal antibodies, indicate that the binding of AO–E and Ca–E is mediated by band 3-related adhesin.

We next examined the effects of chemical modification of AO–E and Ca–E on their binding. AO–E binding was inhibited by DIDS, TNBS, and oxidants, whereas the other amino acid modifiers did not inhibit the binding (Table 1B). Ca–E binding was inhibited by DIDS, DEPC, diamide and sodium periodate (Table 1B). Patterns of the effects of chemical modification on the AO–E binding were the same as those of PE except for NaI/LPO/H_2O_2. In the case of Ca–E, the effects of DIDS, PG, diamide, and sodium periodate were the same as those of PE. The effects of chemical modification on the binding of PE and uninfected cells (especially AO–E) were very similar, suggesting that a common antigenic motif, which plays a role in the PE binding, is the band 3-related adhesin.

DISCUSSION

Erythrocytes parasitized with *P. falciparum* are adhesive due to the presence of several surface adhesins i.e. PfEMP 1, sequestrin, BPORF and modified band 3 protein (reviewed by Sherman *et al.* 1995; Newbold *et al.* 1997). The relative importance each adhesin plays in the binding of the PE to the target cell is, however, unknown. In an attempt to determine the contribution of the modified band 3 protein to PE binding we studied the effects of a variety of chemical modifiers on different isolates, since such infected cells would have differing amounts and organization of PfEMP 1. Further, because there are several pathological conditions wherein erythrocytes become adhesive (i.e. diabetes, thalassemia, sickle cell disease (reviewed by Chappey, Wautier-Pepin & Wautier, 1994)) we reasoned that it might be possible to produce adhesive erythrocytes that were lacking entirely in parasite-encoded adhesins, and to study the effects of chemical modification on these cells. Adhesive erythrocytes were produced by calcium-loading (Ca–E) of normal erythrocytes or acridine orange treatment (AO–E) and the properties of such 'artificial' PE were compared to the natural PE.

Loading of normal erythrocytes with calcium ions in the presence of the ionophore A23187 resulted in adhesive erythrocytes when the calcium concentration was $> 0{\cdot}5$ mM, and with increased levels of calcium up to 2 mM there was a concomitant increase in the number of adhesive cells. These Ca-E bore considerable similarity to PE in that they were immunostained with MAb 3H3, an antibody produced against PE (Crandall & Sherman, 1994*b*), and the binding of these Ca–E was also blocked by this antibody; this is similar to previously reported studies with PE (Crandall & Sherman, 1994*b*). It has been shown that calcium-loading of erythrocytes results in a clustering of membrane proteins including band 3 protein (Lorand *et al.* 1978; Murthy *et al.* 1994); in addition, Ca–E were shown to have proteolysed band 3 and glycophorin (Lorand *et al.* 1983) and a redistribution of phospholipids in the bilayer (Williamson *et al.* 1992).

By treating freshly drawn human erythrocytes with AO it was possible to produce significant numbers of adhesive cells. As with calcium loading, the degree of adhesivity was concentration dependent. These AO–E were immunostained with MAb 3H3, and binding to C32 amelanotic melanoma cells

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was also inhibited by this antibody, but not by Mab B6, an antibody that neither reacts specifically with PE nor blocks PE adherence (Guthrie *et al.* 1995*b*). Though both AO–E and Ca–E show a clustering of band 3 protein (Lelkes *et al.* 1983; Low & Waugh, 1985) their morphologies were distinguishable.

DIDS is a specific inhibitor of anion transport in the erythrocyte, and this effect is due to the covalent cross-linking of lysines 539 and 851 of band 3 protein (Okubo *et al.* 1994). The effect of DIDS on the binding properties of FCR-3, B8B6 and CS2 line was very similar. Indeed, the effect of DIDS on PE binding resembled that of AO–E and Ca–E. These findings suggest that exposure of the band 3 adhesin makes a significant contribution to binding. By the use of radioactive iodine in conjunction with LPO/H_2O_2, it was possible to radio-isotope label PfEMP 1 (Baruch *et al.* 1996). Presumably the iodination of PfEMP 1 does not abolish the binding capacity of this protein since Baruch *et al.* (1996) have shown that a tryptic digest of surface-iodinated PE mimics the properties of PfEMP 1 and binds to CD36, thrombospondin and intercellular adhesion molecule-1. However, as reported earlier (Crandall, Demers & Sherman, 1996), and confirmed herein, the surface iodination of PE strongly inhibited their adherence to C32 amelanotic melanoma cells. Such an effect was evident no matter the organization or amount of PfEMP 1 i.e. it was not isolate-specific. This suggests that a common adhesin might be responsible for PE binding. Since it is known that the inhibitory activity of the adhesive motif in the band 3-related adhesin, HPLQKTY, is ablated by iodination of its tyrosine residue (Crandall *et al.* 1993), it would appear that the common adhesin is related to band 3 protein. Although it is tempting to suggest that AO–E and Ca–E bind by similar mechanisms as the PE this is clearly not demonstrated by chemical modification of tyrosyl residues. One possible explanation is that the tyrosine residue was protected from the iodination in the case of AO–E and Ca–E; alternatively one could postulate that red blood cell adhesivity can be achieved by several mechanisms. In support of this latter mechanism is our finding that AO–E bound to thrombospondin, a known EC receptor for PE, whereas Ca–E did not (S. Eda, unpublished observations).

TNBS is a chemical modifier of lysyl residues (Means, Congdon & Bender, 1972), and treatment of PE with this reagent blocked binding. This effect is also consistent with substitution of the lysyl residue in the adhesive motif of band 3 protein, HPLQKTY, which abolished its inhibitory activity for PE binding (Guthrie *et al.* 1995*a*). TNBS blocked AO–E binding in a fashion similar to PE. DEPC and PG which have been postulated to modify His 819 (Hamasaki, Okubo & Kang, 1992) and Arg 490/730 (Zaki, Böhm & Merckel, 1996) residues of band 3 protein respectively did not inhibit PE binding suggesting that these residues are not involved in adhesion (recall that the band 3-related adhesin involves residues 539-546 (Crandall *et al.* 1993)).

Oxidants have been suggested to be responsible for clustering of band 3 in sickle cells (Schwartz *et al.* 1987) and thalassemic red cells (Rice-Evans, Johnson & Flynn, 1980); oxidative cross-linking has also been postulated to generate the senescence antigen. Possibly, clustering distorts the aggregated proteins causing them to display a conformation not found in the normal erythrocyte. Another explanation is that clustering removes an integral protein from a protected or occluded location thus exposing a normally cryptic site. However, we found that treatment of normal human erythrocytes with the oxidants diamide, sodium periodate, hydrogen peroxide, did not result in adhesive erythrocytes, and phenylhydrazine, and tert-butyl hydroperoxide which did result in adhesive erythrocytes were not immunostained with MAb 3H3. Thus, all of the oxidant treatments did not produce erythrocytes which mimic PE. On the other hand, the oxidants diamide, sodium periodate and ADP/Fe^{3+} did block the binding of PE albeit to varying degrees. Diamide also affected the binding of AO–E and Ca–E, whereas sodium periodate ablated only the binding of Ca–E, and ADP/Fe^{3+} blocked the binding of PE and AO–E, but not Ca–E. Such findings again suggest that while AO–E and Ca–E share properties with PE these 'artificial' PE are not identical to the natural PE. It is difficult to determine the precise mechanism whereby the oxidants affect adherence since the kind of damage inflicted is concentration dependent, and can be highly variable with each oxidant. For example, diamide at 5 mM cross-links spectrin and other proteins (Mohandas *et al.* 1982; Maeda *et al.* 1983), and there is influx of calcium (Parker, 1987). Periodate results in lipid oxidation, oxidation of surface sialyl residues, membrane lipids and sulfhydryls of the membrane protein (Beppu & Kikugawa, 1987). ADP/Fe^{3+} results in oxidation of membrane lipids (Beppu, Ochiai & Kikugawa, 1987; Ando, Beppu & Kikugawa, 1995) and clustering of band 3 (Beppu *et al.* 1990). Possibly aldehydes produced by lipid peroxidation reacted with the lysyl residue of band 3 protein and this led to a block of PE binding; in support of this is the finding that pretreatment of PE bearing the B8B6 line with malondialdehyde ablated binding (data not shown).

AO–E contained echinocytic and shrunken cells without protuberances. Both these types of cells were observed in the AO–E which bound to C32 cells (data not shown). Further, when the calcium was omitted from the Ca-loading solution, the treated erythrocytes showed an echinocytic morphology (data not shown), but did not bind to C32 cells (at 0 mM of calcium). Thus, erythrocyte morphology seems not to be essential to adherence

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mediated by the band 3-related adhesin. This is consistent with the present observation that there was no difference between the effects of chemical modification on the binding of FCR-3 (knobby line) and B8B6 (knobless line).

The mechanisms that result in erythrocyte adhesivity are complex, and modifications in proteins, lipids and saccharides, as well as the synthesis of adhesive ligands, can lead to a 'sticky' erythrocyte. In the case of the PE, the evidence for the involvement of parasite-encoded proteins in binding is not in dispute, however, as the present work shows modified band 3 protein is also a contributor: AO–E and Ca–E, both lacking in parasite-encoded adhesins, mimic to a reasonable degree the modification in binding properties of the PE by chemical agents, and such 'artificial' PE react to 3H3 MAb in a very similar fashion.

Although the properties of AO–E and Ca–E do differ in some respects from one another and from the PE these 'artificial' PEs may be useful models for studying the exposure of the band 3-related adhesin on the PE surface. Further investigations using these models are in progress.

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