Antibodies to sequences in a non-repeat region of *Plasmodium falciparum* antigen Pf155/RESA inhibit either cytoadherence or parasite growth *in vitro*

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SUMMARY

Antibodies to a non-repeat region of the *Plasmodium falciparum* antigen Pf155/RESA were investigated for their capacity to inhibit parasite cytoadherence to melanoma cells and parasite growth *in vitro*. The activities of these antibodies were studied since the target region in Pf155/RESA includes a cytoadherence-related motif also found in loop 3 and 7 of human erythrocyte band 3 protein. Overlapping multiple antigen peptides (MAPs) together spanning residues 199–220 of Pf155/RESA were used to raise antibodies in rabbits. Analysis of the fine specificity of these antibodies revealed that antibodies raised against largely overlapping sequences displayed highly different specificity patterns. Similarly, striking differences were seen when analysing the biological effect of antibodies to these MAPs. Antibodies to the cytoadherence-related motif of Pf155/RESA, as well as antibodies raised against a MAP based on a corresponding band 3 motif, inhibited cytoadherence but not parasite growth. In contrast, antibodies to sequences adjacent to the Pf155/RESA cytoadherence motif inhibited parasite growth *in vitro* but had no effect on cytoadherence.

Key words: *Plasmodium falciparum*, Pf155/RESA, RBC band 3, cytoadherence.

INTRODUCTION

In *Plasmodium falciparum* infections in humans cytoadherence of infected red blood cells (RBC) to endothelial cells is responsible for sequestration and is believed to play an important role in the pathogenesis of cerebral malaria. PfEMP1 appears to be the major parasite-associated protein mediating cytoadherence (Baruch *et al*. 1996), but experimental data indicate involvement also of other proteins in this phenomenon (Berendt, Ferguson & Newbold, 1990).

Pf155/RESA is a 155 kDa protein associated with dense granules in the apical part of merozoites and is translocated to the RBC membrane during or shortly after invasion (Aikawa *et al*. 1990). The antigen is associated with the cytoskeleton on the cytoplasmic side of the membrane via binding involving spectrin (Foley *et al*. 1991; Ruangjirachuporn *et al*. 1991) and it appears not to be exposed on the outer surface of the *Plasmodium*-infected RBC (PRBC) (Berzins, 1991). Pf155/RESA contains 2 conserved regions of tandemly repeated sequences, 1 in the C-terminus and 1 in the middle of the molecule (Favaloro *et al*. 1986). A sequence homology between a hexapeptide motif in the N-terminal region of Pf155/RESA (residues 213–218) and the cytoadherence-related motifs in RBC band 3 has been reported previously (Iqbal *et al*. 1995). Synthetic peptides corresponding to these motifs inhibit PRBC cytoadherence to C32 melanoma cells *in vitro* (Iqbal *et al*. 1995).

In the present study we investigated if antibodies to the cytoadherence-related motif of Pf155/RESA may inhibit PRBC binding to melanoma cells in the same manner as antibodies to the band 3 motif. Furthermore, as antibodies to both repeat and non-repeat sequences of Pf155/RESA inhibit *P. falciparum* growth *in vitro* (Berzins *et al*. 1986; Wåhlin *et al*. 1992; Siddique *et al*. 1998) we investigated if antibodies to the cytoadherence-related motif and to sequences adjacent to this motif also show such inhibitory activity. The antibodies used were generated in rabbits by immunization with multiple antigen peptides (MAPs) and were analysed with regard to fine specificity and reactivity with Pf155/RESA and band 3.

MATERIALS AND METHODS

Synthetic peptides

Six multiple antigen peptides (MAPs) based on sequences derived from loops 3 and 7 in human RBC band 3 or a non-repeat region of Pf155/RESA (Table 1) were synthesized by solid-phase Fmoc chemistry essentially as previously described (Ahlborg, 1995; Sällberg *et al*. 1991). Briefly,

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Table 1. MAP sequences used from Pf155/RESA and band 3

MAP	Antigen	Residues	Amino acid sequence
MAP14	Pf155/RESA	199–211	IMDINKRKYDSLK
MAP15	Pf155/RESA	202–214	INKRKYDSLKEKL
MAP16	Pf155/RESA	207–221	YDSLKEKLQKTYSQY
MAP18	Pf155/RESA	211–220	KEKLQKTYSQ
MAP11	Band3 loop3	546–555	DHPLQKTYNY
MAP12	Band3 loop7	821–834	DVPYVKRVKTWRMH

amino acids pre-activated as Opfp- or DHBT (Ser and Thr) esters (NovaBiochem, Läufelingen, Switzerland) were used for the coupling to Polyhipe PR500 resin (0·3 mmol/g). Double couplings with a 4 × excess of amino acids were used throughout the synthesis. Tetrameric MAPs were assembled by 2 consecutive couplings of Fmoc-Lys-(Fmoc)-Opfp followed by a straightforward synthesis of the linear peptide branches. After cleavage from the resin and removal of the side-chain protection groups, C-terminally amidated MAPs were precipitated and washed in ice-cold diethylether followed by lyophilization. Amino acid analysis of the MAPs was in good agreement with the expected composition.

Rabbit antisera and preparation of IgG

Each MAP was used to immunize 1 New Zealand white rabbit by intramuscular injections with 100 μg of peptide in phosphate-buffered saline (PBS) emulsified 1 : 1 in Freund's complete adjuvant (FCA; Difco, Detroit, MI, USA). Booster injections were given with Freund's incomplete adjuvant (FIA) at week 3, 8 and 14 respectively. The rabbits were bled 1 week after each booster injection. IgG was isolated on Protein-A Sepharose (Pharmacia, Uppsala, Sweden) from the sera taken 1 week after the last booster injection.

Peptide ELISA

MAPs were used for coating EIA/RIA high-binding plates (Costar, Cambridge, MA) at a concentration of 5 μg/ml in PBS. ELISA was performed as described previously (Ahlborg, 1995) using goat anti-rabbit IgG conjugated to alkaline phosphatase (Mabtech AB, Stockholm, Sweden) for detection. Antibody titres were estimated by analysing serial dilutions of sera for reactivity with the MAP used for immunization, using an absorbance of 0·5 at 405 nm as a cut-off value.

PEPSCAN

Overlapping octamer peptides were synthesized on polyethylene pins by Fmoc chemistry according to the instructions of the manufacturer (Cambridge Research Biochemicals, Cambrige, UK) (Geysen *et al*. 1987). The N-terminals of the peptides were acetylated. Octapeptides with 1 amino acid overlap, together corresponding to band 3 residues 539–569 (loop 3) and 814–834 (loop 7) (Tanner, Martin & High, 1988) as well as Pf155/RESA residues 191–227 (Favaloro *et al*. 1986) were synthesized. Rabbit antisera at a dilution of 1 : 2000 were incubated with the pins and bound antibodies were detected as previously described (Iqbal *et al*. 1995).

Immunofluoresence

Antibody reactivity with antigens associated with the membrane of *P. falciparum* PRBC or with the intraerythrocytic parasite was analysed on methanol-fixed or only air-dried monolayers of PRBC, respectively (Crandall & Sherman, 1991). Antibodies to be tested were added in serial dilutions and incubated for 30 min. Bound antibodies were detected by biotinylated goat anti-rabbit IgG followed by fluorescein-conjugated avidin (Vector Laboratories Inc., Burlingame, CA, USA). Fixed slides were counter-stained with ethidium bromide to visualize intraerythrocytic parasites (Perlmann *et al*. 1984).

Parasites

P. falciparum laboratory strains used were the Gambian strain FCR3 and the Ugandan strain Palo Alto (kindly provided by Dr Odile Mercereau-Puijalon). Cultures were maintained as described by Trager & Jensen (1976).

Cytoadherence assay

The cytoadherence assay was performed as previously described (Rogers *et al*. 1996) with modifications. Briefly, $2{\cdot}5 \times 10^3$ C32 melanoma cells (American Type Culture No. CRL 1585) per well were seeded into 8-well Lab-Tek chamber slides (Nunc, Naperville, IL, USA) and cultured for 48 h at 37 °C at 5 % CO_2 using RPMI-1640 with 10 % fetal calf serum (FCS) as medium. The cells were fixed with 1 % formaldehyde in PBS, pH 7·2, for 1 h at room temperature and stored in sterile PBS at 4 °C until use. Shortly before the addition of Palo Alto PRBC the wells were washed twice with binding medium (BM; RPMI-1640 plus 25 mM HEPES, 10 % FCS, pH 6·8, and no bicarbonate) and then

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incubated with BM for 20 min at 37 °C. The BM was removed and serial dilutions of either MAPs or anti-MAP IgGs were added to the wells immediately before the addition of 0·2 ml of late-stage infected PRBC at a 2% haematocrit. The chamber slides were then incubated at 37 °C for 1·5 h on a rocking table. Chambers were removed and the slides were rinsed 4 times with BM at 37 °C. The PRBCs bound to melanoma cells were fixed with 1% glutaraldehyde in PBS, air dried and stained with 5% Giemsa. The number of PRBCs attached to 500 C32 cells was recorded by light microscopy. Each sample was tested in duplicate in at least 3 independent assays. The percentage of adhesion inhibition was calculated as 100 × (mean of PRBCs bound to C32 cells in control wells − mean of PRBCs bound to C32 cells in test wells/mean PRBCs bound to C32 cells in control wells). Control wells were where the PRBCs were allowed to bind to C32 cells in the absence of MAPs or antibodies to MAPs.

Parasite growth inhibition in vitro

Parasite cultures were synchronized by sorbitol treatment (Lambros & Vanderberg, 1979) and were grown to contain mainly late trophozoites or early schizonts. Parasite cultures (Palo Alto) at a final parasitaemia of 1% and a haematocrit of 2% were set up in microculture plates (Wåhlin *et al*. 1984). The cultures were mixed with malaria culture medium supplemented with serial dilutions of different IgGs and were incubated at 37 °C for 20 h by the candle jar method. The cells were harvested and stained for analysis in the Coulter Epics XL MCL flow cytometer (Coulter, Miami, FL, USA). The percentage parasite growth inhibition by antibodies was calculated as 100 × (percentage parasitaemia in the control − percentage parasitaemia in the test)/(percentage parasitaemia in the control). Duplicates were set up for all samples and the percentages of parasitaemia were determined from the mean of 20000 RBC analysed.

RESULTS

MAP based on sequences derived either from a non-repeat region of Pf155/RESA including a cytoadherence related motif (Iqbal *et al*. 1995) or from the corresponding motifs in loops 3 and 7 of human RBC band 3 (Table 1) were synthesized and used to immunize rabbits. After 4 injections, almost all MAPs had elicited antibody titres of $2–20 \times 10^3$ as measured by ELISA using the homologous MAP as antigen. One exception was MAP11, based on loop 3 of band 3, which did not induce any detectable antibody response although several rabbits were injected repeatedly.

The fine specificity of the antibodies elicited by the different MAPs was analysed by PEPSCAN–ELISA using overlapping octapeptides corresponding to the relevant sequences in Pf155/RESA and loop 7 of band 3 (Fig. 1). All antisera displayed a distinct reactivity with sequences contained within the MAP used for immunization. Interestingly, the fine specificity of the antibodies elicited by largely overlapping peptides differed markedly. For example, MAP15 overlapped MAP14 with 10 out of 13 amino acids, still, antibodies to them recognized largely different sequences as did antibodies to the partially overlapping MAP15 and 16. Similarly, MAP18 which is a part of the sequence in MAP16 gave rise to antibodies of a different specificity than did MAP16 although the recognized sequences overlapped. Antibodies to MAP12, 16 and 18 all reacted with peptides implicated in the cytoadherence related motifs of band 3 (MAP12) and Pf155/RESA (MAP16 and 18), respectively (Iqbal *et al*. 1995; Crandall & Sherman, 1994). However, the antibodies to the Pf155/RESA sequences did not cross-react with band 3 peptides and neither did the antibodies to MAP12 (band 3 loop 7) react with the Pf155/RESA sequences. Furthermore, none of these antibodies showed any cross-reactivity with the sequences representing the cytoadherence-related region of loop 3 (not shown). The low degree of cross-reactivity of the anti-MAP antibodies was also reflected in results from ELISA where the reactivity of the antisera was tested against all the different MAPs (Fig. 2). The only marked cross-reactivity was exhibited by anti-MAP15 antibodies with MAP14 whereas anti-MAP14 antibodies reacted weakly with MAP15. Cross-reactivity between antibodies to MAP16 and 18 was also rather low despite the fact that the sequence of MAP18 is completely represented within the MAP16 sequence. One interpretation of this could be that structural restraints within the peptides, and not only the primary sequence, affect antibody recognition. None of the antisera showed any significant reactivity with MAP11 based on the cytoadherence-related motif of loop 3 of band 3 (not shown).

The reactivity of the anti-MAP antibodies with PRBCs was analysed by means of indirect immunofluorescence using either methanol-fixed or unfixed PRBC (Table 2). IgG from all antisera gave a weak membrane staining of fixed PRBCs with early stages of the parasite, while antibodies to MAPs containing the cytoadherence-related motifs of Pf155/RESA (MAPs 16 and 18) or band 3 (MAP12) also gave a punctated pattern of staining of PRBCs infected with late stages of the parasite (data not shown). Thus, despite the fact that antibodies to MAP16 and 18 do not recognize band 3 peptides in ELISA (Figs 1 and 2), a possible cross-reactivity with band 3 is indicated by immunofluorescence. Using unfixed air-dried PRBCs, only antibodies to MAPs 14 and 15 displayed smooth staining covering the whole pigmented PRBCs (not shown). None of the antisera

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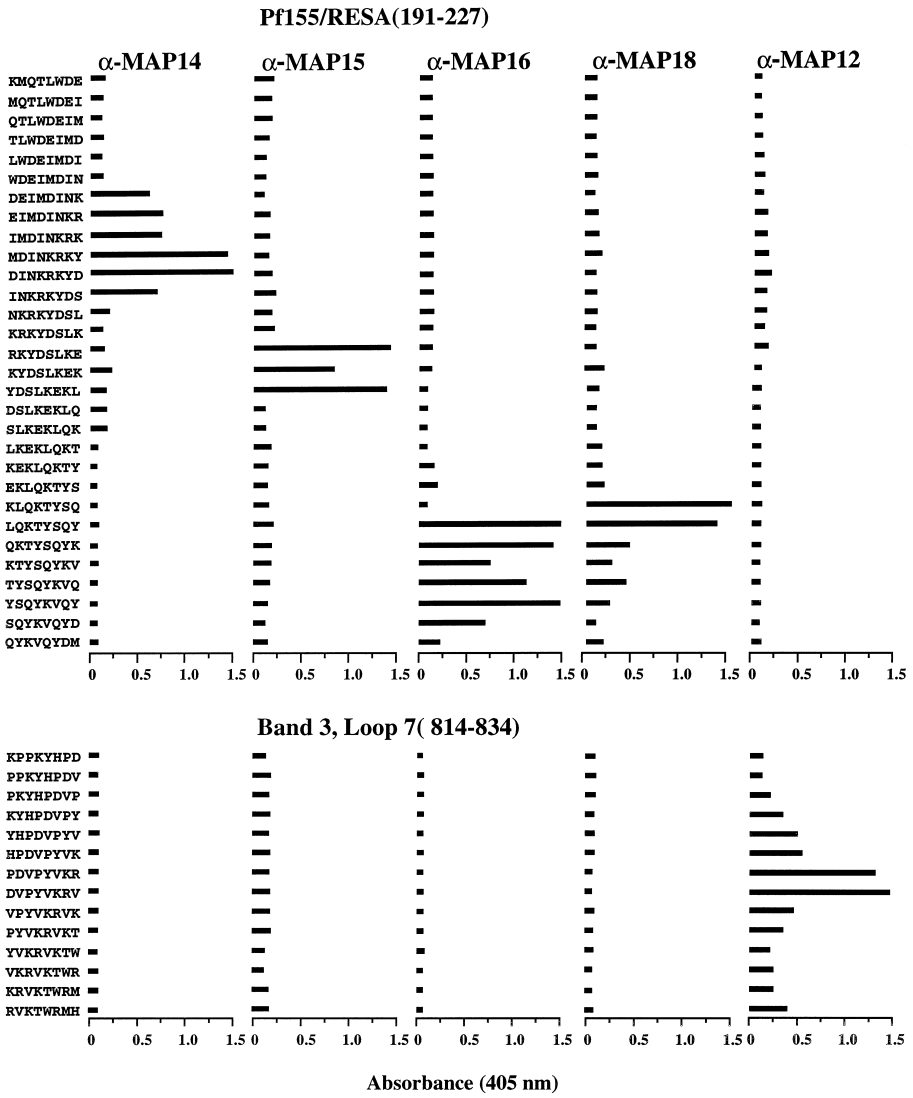

Fig. 1. PEPSCAN–ELISA analysis of rabbit antisera to MAP immunogens based on sequences in Pf155/RESA (MAP14, 15, 16 and 18) and band 3 (MAP12). The antibody reactivity was analysed to octapeptides with 1 amino acid overlap covering residues 191–227 of Pf155/RESA and 814–834 of loop 7 in band 3 as indicated on the *y*-axis. Absorbance at 405 nm is shown on the *x*-axes.

showed reactivity with fixed or unfixed normal RBCs. No immunofluorescence was detected with IgG from any of the pre-immune sera.

In order to investigate if antibodies elicited by the Pf155/RESA or band 3 based MAPs had parasite neutralizing properties, purified IgG from these antisera were analysed for their capacity to interfere with parasite growth *in vitro*. Only antibodies to Pf155/RESA sequences (MAPs 14 and 15) inhibited parasite growth (Table 3). In contrast, antibodies to sequences including the cytoadherence-associated motifs of either Pf155/RESA (MAPs 16 and 18) or band 3 (MAP12) showed, like IgG from all pre-immune sera, no significant effect on parasite growth (Table 3).

Peptides corresponding to the cytoadherence-associated motifs in band 3 have previously been shown to inhibit PRBC binding to C32 melanoma cells (Crandall *et al*. 1993) as do peptides containing the homologous motif in Pf155/RESA (Iqbal *et al*. 1995). To ascertain that these sequences retained their cytoadherence-inhibitory capacity when included as parts in to MAP constructs, binding of PRBCs to melanoma cells in the presence of MAPs was analysed. The results were in line with those obtained previously with linear peptides, MAPs comprising the cytoadherence-related motifs being inhibitory in a dose-dependent manner (MAP11, 12, 16 and 18) while the 2 MAPs not including this motif (MAP14 and 15) had no effect on cytoadherence (Fig. 3).

Antibodies to the cytoadherence-related motifs of band 3 have previously been shown to inhibit the binding of PRBCs to melanoma cells (Crandall & Sherman, 1994). In order to see if antibodies to the corresponding motif in Pf155/RESA show a similar inhibitory activity, the IgG fractions of antisera to the MAPs were analysed for their capacity to inhibit

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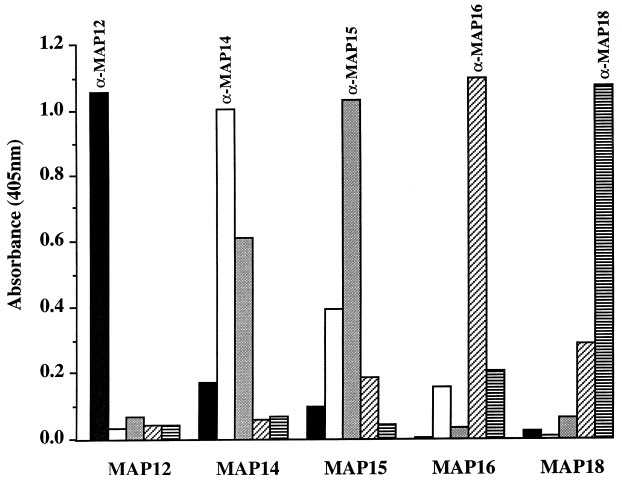

Fig. 2. Analysis by ELISA of the cross-reactivity of different anti-MAP antisera (1:1000) with other MAPs. The MAP used as antigen is indicated below each group of bars and the identity of the antibody reactivity which the bars represent is indicated above the bars. Absorbance at 405 nm is shown on the *y*-axis.

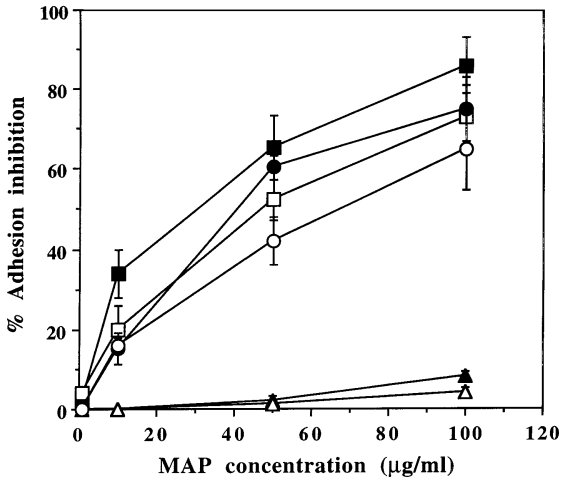

Fig. 3. Inhibition of cytoadherence of PRBC (Palo Alto strain) to C32 melanoma cells with band 3 and Pf155/RESA MAPs. The mean values obtained from 3 experiments with duplicates in each experiment are presented. MAPs used were MAP11 (■) and MAP12 (□) from band 3 and MAP14 (▲), MAP15 (△), MAP16 (●) and MAP18 (○) from Pf155/RESA.

Table 2. Immunofluorescence reactivity of rabbit pre-immune (PI) and immune (I) IgG to MAPs with PRCBs

Rabbit antiserum	IgG used	Minimal IgG (μg) required for positive reactivity in: Fixed — Ring	Fixed — Late	Unfixed — Late
Anti-MAP14	PI	—	—	—
	I	52	—	52
Anti-MAP15	PI	—	—	—
	I	40	—	40
Anti-MAP16	PI	—	—	—
	I	42	42	—
Anti-MAP18	PI	—	—	—
	I	36	36	—
Anti-MAP12	PI	—	—	—
	I	40	40	—

binding of PRBCs to melanoma cells *in vitro*. Like antibodies to the band 3 motif (MAP12), antibodies to the homologous sequence in Pf155/RESA (MAP16 and 18) inhibited the cytoadherence of PRBCs at a similar level, 56, 48 and 52%, respectively (Table 3). On the other hand, antibodies to Pf155/RESA sequences adjacent to the cytoadherence-related motif (MAP14 and 15), had no inhibitory effect on cytoadherence, neither had any of the IgG fractions from the pre-immune sera.

DISCUSSION

We previously reported that a hexapeptide motif in a non-repeat region of Pf155/RESA (residues 213–218, KLQKTY), is highly homologous to a consensus cytoadherence-inhibitory motif found in loop 3 (547–553) and loop 7 (829–824) of human RBC band 3 (Iqbal *et al*. 1995). Interestingly, synthetic peptides containing this Pf155/RESA sequence

Table 3. Cytoadherence and parasite growth inhibition of PRCBs with rabbit pre-immune (PI) and immune (I) IgG to MAPs

Rabbit serum	IgG used	IgG conc. (μg/ml)	Cytoadherence inhibition (% ± S.D.)	Parasite growth inhibition (% ± S.D.)
Anti-MAP14	PI	600	0 ± 0	0 ± 0
	I	650	0 ± 0	46 ± 10
Anti-MAP15	PI	600	0 ± 0	3 ± 1
	I	500	0 ± 0	29 ± 7
Anti-MAP16	PI	480	4 ± 1	4 ± 2
	I	520	48 ± 12	4 ± 1·1
Anti-MAP18	PI	400	5 ± 2	4 ± 1
	I	450	52 ± 15	4 ± 2·3
Anti-MAP12	PI	300	6 ± 2	4 ± 1·4
	I	500	56 ± 12	4 ± 2

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Table 4. Homologies between sequences in Pf155/RESA, RESA2 and band 3

Antigen	Residues	Amino acid
Band3 Loop7	829–824*	WRMHTK-VRKVYPVD
		: : I I
Band3 Loop3	547–553	DHPLQKTYNY
		: IIIII
Pf155/RESA	199–221	IMDINKRKYDSLKEK-LQKTYSQY
		IIIIII:II II I I I I I
RESA2	133–156	IMDINKKKYTVLKAK-LRKKYHKY

* Band 3 Loop 7 sequence is written from C-terminus to N-terminus.

inhibited PRBC cytoadherence to melanoma cells *in vitro* (Iqbal *et al*. 1995), as was also shown previously with peptides containing the band 3 derived sequences (Crandall *et al*. 1993). MAPs presenting these sequences were somewhat less efficient in inhibiting cytoadherence as compared with the corresponding linear peptides (Iqbal *et al*. 1995), which is to be expected as engagement of 1 of the branches of a MAP in binding limits the availability of the other branches to bind to other sites due to steric reasons. Band 3-reactive antibodies directed against these peptide motifs also have the capacity to inhibit binding of PRBC to melanoma cells (Crandall & Sherman, 1994). We show herein that antibodies to the corresponding Pf155/RESA motif may also interfere with such PRBC cytoadherence, while antibodies to adjacent sequences are without effect. In contrast, antibodies to the latter sequences inhibited parasite growth in *P. falciparum in vitro* cultures, while antibodies to the cytoadherence-related motif were inefficient.

The reason why antibodies to 2 adjacent sequences in Pf155/RESA have such differential anti-parasitic activities is not known. A plausible explanation for this could be that Pf155/RESA itself is not a target for the antibodies in the parasite growth inhibition or the cytoadherence inhibition, but that the antibodies cross-react with other antigens. Since Pf155/RESA is a sub-membraneous protein associated with the cytoskeleton on the cytoplasmic aspect of the RBC membrane (Foley *et al*. 1991; Ruangjirachuporn *et al*. 1991) and appears not to be exposed on the RBC surface (Berzins, 1991), it is difficult to foresee any direct involvement of Pf155/RESA in binding of PRBCs to endothelial cells. However, Pf155/RESA is also found abundantly in supernatants from *P. falciparum* cultures (Carlsson *et al*. 1991), indicating that the antigen may also be present at other as yet not identified locations. Nevertheless, for the cytoadherence inhibitory anti-Pf155/RESA antibodies the homologous motifs in band 3 are obvious alternative target structures (Crandall *et al*. 1993; Iqbal *et al*. 1995). Cross-reactivity of antibodies to the Pf155/RESA cytoadherence motif with band 3 is actually indicated by the immunofluorescence results although the specificity analyses with peptides did not suggest such cross-reactivity. Still, antibody reactivity with one and the same sequence in a protein and in a peptide does not only depend on the primary structure but also on conformational restraints depending on adjacent amino acids.

Antibodies to the repeat sequences of Pf155/RESA are efficient in inhibiting merozoite invasion *in vitro* (Berzins *et al*. 1986; Wåhlin *et al*. 1992), as are antibodies to non-repeated sequences in the N-terminal part of the antigen (Siddique *et al*. 1998). For the parasite inhibitory effects of antibodies to repeats, both Pf155/RESA and cross-reactive antigens appear to be targets (Wåhlin *et al*. 1992). However, antibodies to non-repeat sequences of the antigen may also inhibit the *in vitro* growth of Pf155/RESA deficient parasites (Siddique *et al*. 1998), suggesting the presence of an antigen exhibiting a high degree of homology with Pf155/RESA. A possible candidate antigen in this context would be RESA-2, which is highly homologous to Pf155/RESA, but lacks the repeat regions of this antigen (Cappai *et al*. 1992). A higher degree of homology with the corresponding RESA-2 sequence of the Pf155/RESA sequences included in MAPs 14 and 15 than those presented in MAPs 16 and 18 (Table 4) could explain the differential parasite growth-inhibitory activity of antibodies to these MAPs. However, expression of RESA-2 has as yet only been detected at the mRNA level and not at the protein level (Vazeux, Le Scanf & Fandeur, 1993), although antibodies to non-repeat sequences of Pf155/RESA give immunofluorescence also with parasites deficient in this antigen (Siddique *et al*. 1998).

Although the peptide sequences in different MAPs largely overlapped or showed a high degree of homology, the antibodies were remarkably specific for the sequence which induced them. However, the antibodies generally also showed a significant, although weak, cross-reactivity with the native protein. Such low cross-reactivity of MAP-induced antibodies with related MAPs, while they exhibit a consistent reactivity with the native protein has been observed previously (Ahlborg, 1995). The weak

reactivity with the native protein displayed by antibodies raised against MAP may have several explanations. It is possible that only a minor fraction of the anti-MAP antibodies actually recognize the corresponding protein, a common finding when synthetic oligopeptides are used as immunogens (Berzins *et al*. 1986). The unique non-repeated sequences in the native protein may show a restricted flexibility in secondary structure as compared with the corresponding sequences in MAPs. Furthermore, these sequences in the native protein may show a limited accessibility for antibody binding.

MAP11 based on the cytoadherence related motif of loop 3 in band 3 failed to elicit detectable antibody responses. However, the immunogenicity in rabbits of this band 3 sequence, using MAP immunogens has been demonstrated by others, who, however, found MAPs based on loop 7 (821–834) to be less immunogenic (Crandall & Sherman, 1994). The discrepancy between the 2 studies may be due to minor differences of the MAP constructs or to the fact that the rabbits used are not inbred and thus can display large individual variation. Furthermore, the loop 3 sequence is recognized by naturally acquired antibodies in individuals living in malaria endemic areas (Hogh *et al*. 1994), indicating that the sequence as presented in band 3 is immunogenic in humans. However, as malaria-immune individuals also show antibodies reactive with sequences including the homologous motif of Pf155/RESA (Kulane *et al*. 1997), it is possible that the reactivity seen against the band 3 motif is due to cross-reactive antibodies elicited by Pf155/RESA.

Our results show that the segment 199–221 of Pf155/RESA contains sequences with potential to induce both parasite growth inhibitory antibodies and antibodies with capacity to interfere with parasite sequestration. The N-terminally adjacent sequence 176–199 contains T-cell epitopes frequently recognized by lymphocytes from malaria-immune humans (Rzepczyk *et al*. 1988; Troye-Blomberg *et al*. 1989; Kulane *et al*. 1997). Thus, the segment 176–221 of Pf155/RESA should constitute an immunogen of interest for vaccine development, having the potential both to elicit antibodies with dual anti-parasitic activities as well as to induce an immunological memory.

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