

Mapping of the Region of Complement Receptor (CR) 1 Required for *Plasmodium falciparum* Rosetting and Demonstration of the Importance of CR1 in Rosetting in Field Isolates¹

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The malaria parasite *Plasmodium falciparum* induces a number of novel adhesion properties in the erythrocytes that it infects. One of these properties, the ability of infected erythrocytes to bind uninfected erythrocytes to form rosettes, is associated with severe malaria and may play a direct role in the pathogenesis of disease. Previous work has shown that erythrocytes deficient in complement receptor (CR) 1 (CR1, CD35; C3b/C4b receptor) have greatly reduced rosetting capacity, indicating an essential role for CR1 in rosette formation. Using deletion mutants and mAbs, we have localized the region of CR1 required for the formation of *P. falciparum* rosettes to the area of long homologous repeat regions B and C that also acts as the binding site for the activated complement component C3b. This result raises the possibility that C3b could be an intermediary in rosetting, bridging between the infected erythrocyte and CR1. We were able to exclude this hypothesis, however, as parasites grown in C3-deficient human serum formed rosettes normally. We have also shown in this report that rosettes can be reversed by mAb J3B11 that recognizes the C3b binding site of CR1. This rosette-reversing activity was demonstrated in a range of laboratory-adapted parasite strains and field isolates from Kenya and Malawi. Thus, we have mapped the region of CR1 required for rosetting and demonstrated that the CR1-dependent rosetting mechanism occurs commonly in *P. falciparum* isolates, and could therefore be a potential target for future therapeutic interventions to treat severe malaria. *The Journal of Immunology*, 2000, 165: 6341–6346.

Complement receptor (CR)³ 1 (CR1, CD35, C3b/C4b receptor) is an immune regulatory molecule that is expressed on the surface of erythrocytes, some peripheral blood leukocytes, glomerular podocytes, and follicular dendritic cells (1). CR1 binds to the activated complement components C3b and C4b, and thereby brings about a number of important functions including clearance of immune complexes from the circulation, enhancement of phagocytosis, and regulation of complement activation by means of decay-accelerating activity for C3 converta-

ses and cofactor activity for factor I (1). CR1 is composed of a number of repeated domains called short consensus repeats (SCRs, sometimes known as complement control protein repeats), each of which is composed of ~60 amino acids containing four invariant cysteines and a number of other conserved residues (2). The extracellular domain of the most common allelic variant of CR1 is composed of 30 SCRs, the first 28 of which are arranged in tandem repeats in homologous groups of 7, with each group of 7 being known as a long homologous repeat (LHR) (Ref. 3; see Fig. 1A). The binding sites on CR1 for its ligands C3b and C4b have been mapped, with SCRs 8–12 and SCRs 15–18 preferentially binding to C3b and SCRs 1–4 preferentially binding to C4b (Refs. 3–5; see Fig. 1A).

The ability of erythrocytes infected with *Plasmodium falciparum* to bind uninfected erythrocytes to form rosettes is a property shown by only some parasite isolates, but is of importance because it has been associated with severe malaria in many studies (e.g., Refs. 6 and 7). We have shown previously that CR1 on uninfected erythrocytes is required for the formation of rosettes in some laboratory-adapted parasite strains, as CR1-deficient erythrocytes showed reduced rosetting and soluble recombinant CR1-inhibited rosetting (8). We also demonstrated that erythrocytes with a CR1 polymorphism that is common in African-Americans showed reduced binding to the parasite rosetting ligand *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), and we hypothesized that this polymorphism may have been selected for in malarious regions by providing protection against severe malaria (8). The latter hypothesis would only be valid if CR1-mediated rosetting occurs in natural parasite isolates, but to date, there have been no studies addressing the CR1 dependence of rosetting in field isolates.

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³ Abbreviations used in this paper: CR, complement receptor; LHR, long homologous repeat; PfEMP1, *P. falciparum* erythrocyte membrane protein 1; SCR, short consensus repeat.

This study set out to further characterize the role of CR1 in rosetting by identifying the region of CR1 required for interaction with infected erythrocytes and by determining the requirement for CR1 in the rosetting of *P. falciparum* field isolates.

Materials and Methods

Deletion mutants, Abs, and reagents

The CR1 deletion mutants LHR-A, LHR-B, and LHR-D⁺ were produced from culture supernatants of transiently transfected COS-7 cells as described previously (9–11). LHR-A comprises SCRs 1–7, LHR-B comprises SCRs 8–14, and LHR-D⁺ comprises SCRs 22–30 (Fig. 1A). The first three SCRs of LHR-C (SCRs 15–17) were expressed in *Pichia pastoris* (12) and C3b dimers were produced from human plasma C3 as described elsewhere (11). Soluble recombinant CR1 (13) and mAb YZ-1 were obtained from D. Fearon (University of Cambridge, Cambridge, U.K.). The CR1 mAbs J3B11, J8B10, and J3D3 were as described previously (14); 1B4, 3D9, 7G8, 9H3, and HB8592 were obtained from R. Taylor (University of Virginia School of Medicine, Charlottesville, VA); 1F11.G12, 3C6.D11, and 6B1.H12 were obtained from T Cell Sciences (Needham, MA); and E11 was purchased from Serotec (Oxford, U.K.). Additional amounts of mAb J3B11 were also obtained from J. Cohen (University of Reims, Reims, France). The CD36-specific mAb OKM5 was a gift from T. Mercolino (OrthoDiagnostic Systems, Raritan, NJ), isotype control mAbs were purchased from PharMingen (San Diego, CA) and rabbit Ig (X0936) and rabbit polyclonal Ab to human IgA/G/M (A1090) were purchased from Dako (Carpinteria, CA).

Parasites and parasite culture

The *P. falciparum*-rosetting laboratory strains used were clone R29 (15), clone PAR⁺ (16), and lines TM180 and TM284 (17). Rosetting in these parasite strains is 40–80% of infected erythrocytes in rosettes and is stable within a given cycle, but varies from one cycle to the next. Parasites were cultured in group O human erythrocytes in RPMI 1640 medium with sodium bicarbonate (Life Technologies, Rockville, MD) supplemented with 2 mM L-glutamine, 25 mM HEPES, 20 mM D-glucose, 25 µg/ml gentamicin, and 10% normal human serum (pooled from at least four donors), 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 (18) and were synchronized once a week by sorbitol lysis (19). Field isolates from malaria patients in Kenya and Malawi were collected as described previously (7) and cultured in supplemented RPMI 1640 as above except that 10% pooled human AB serum was used to avoid ABO incompatibility. Isolates were grown for at least 16 h in vitro and maturity assessed by Giemsa-stained thin films. Only those isolates in which the majority of parasites matured into pigmented trophozoites and had a rosette frequency of >10% infected erythrocytes in rosettes were used in Kenya and of >5% infected erythrocytes in rosettes were used in Malawi.

Rosette reversal by CR1 deletion mutants

Parasitised cells were stained with 20 µg/ml ethidium bromide for 5 min at 37°C, washed twice with incomplete RPMI 1640 (supplemented as above but without serum), and resuspended at 4% hematocrit in binding medium (RPMI 1640 medium without sodium bicarbonate, supplemented as above but with 10% pooled human serum that had been heat-inactivated at 56°C for 30 min). Ten microliters of each LHR CR1 deletion mutant was added to a 10-µl aliquot of culture suspension to give a final concentration of 50 µg/ml deletion mutant (~1 µM) and then incubated for 1 h at 37°C and assessed for rosetting as described below. The negative control culture was incubated with 10 µl of COS-7 cell culture supernatant from mock-transfected cells, and the positive control culture was incubated with 50 µg/ml soluble CR1 (~250 nM), which had been shown previously to cause 50% inhibition of rosetting in clone R29 (8). SCRs 15–17 were tested at 50 µg/ml and 500 µg/ml as described above, and rosetting was compared with a control culture with no added recombinant protein.

Assessment of rosetting

Ten microliters of culture suspension, prestained with ethidium bromide as described above, was placed on a clean glass slide and covered with a 22-mm × 22-mm coverslip. Slides were blinded before counting and then were viewed on an epifluorescence microscope using a combination of fluorescence and bright field. The illumination was adjusted so that both ethidium bromide-stained infected erythrocytes and unstained uninfected erythrocytes could be viewed simultaneously. At least 200 infected cells were counted and assessed for rosetting, with the binding of two or more

uninfected erythrocytes constituting a rosette. The rosette frequency is the percentage of mature (pigmented trophozoite) infected erythrocytes forming rosettes.

Rosette reversal by CR1 mAbs

Parasite cultures were stained with 20 µg/ml ethidium bromide as described above and resuspended in binding medium at 2% hematocrit. mAbs were added to 20-µl aliquots of culture suspension to give a final concentration of 1 µg/ml and 10 µg/ml and were incubated at 37°C for 30 min before assessment of rosetting. Isotype controls were tested at the same concentration. mAb J3B11 was also tested at 0.1 µg/ml, 0.5 µg/ml and 100 µg/ml.

Inhibition of rosette reformation by mAbs J3B11, 1B4, and 3D9

The mAbs were added to 100-µl aliquots of R29 culture suspension at 2% hematocrit to give a final concentration of 1 µg/ml; then rosettes were mechanically disrupted by passing the suspension 10 times through a 26-gauge needle (17). A negative control culture with no additive and a culture with 1 µg/ml IgG1 isotype control were treated in the same way. The cultures were incubated at 37°C for 1 h to allow rosettes to reform, then assessed for rosetting as described above.

Inhibition of rosette reformation by C3b dimers

C3b dimers were added to culture suspension at 2% hematocrit to give a final concentration of 100, 10, and 1 µg/ml, then mechanically disrupted, incubated, and assessed for rosetting as above.

Rosetting in C3-deficient serum

Serum from a patient genetically deficient in C3 was obtained from Mark Walport (Imperial College, London, U.K.). Parasites were grown for one full cycle (from mature trophozoite stage through to mature trophozoite stage in the next cycle) in complete RPMI 1640 containing 10% C3-deficient serum or 10% normal human serum, and rosetting was assessed as described above.

Rosette reversal by mAbs and polyclonal Abs in laboratory strains and field isolates

J3B11 and an isotype control were tested at 1 µg/ml, and rosetting was compared with that in a control culture with no added Ab. The CD36 mAb OKM5 and an isotype control were tested at 10 µg/ml, a concentration shown previously to abolish rosetting in the parasite line Malayan Camp (20). The rabbit polyclonal Abs to human IgA/IgG/IgM were tested at 1/50 dilution and compared with rosetting in the presence of an equivalent concentration of Ig derived from normal rabbit serum (1/100 dilution). The latter control was not available for the testing of Kenyan field isolates. All incubations with Ab were for 30 min at 37°C, and assessment of rosetting was as described above. Malawi experiments were conducted by S.J.R., who was supplied with blinded J3B11 and IgG1 isotype control.

Results

Rosette reversal by CR1 deletion mutants

To localize the region of CR1 required for *P. falciparum* rosetting, we tested the ability of soluble CR1 deletion mutants (Fig. 1A) to reverse rosettes with parasite clone R29. A soluble CR1 recombinant protein consisting of the entire extracellular domain of CR1-reversed rosetting (Fig. 1B), as had been shown previously (8), and a deletion mutant consisting of LHR-B also reversed rosetting to ~50% of the control value (Fig. 1B, control mean rosette frequency, 46.5% (SE, 1.4); LHR-B mean rosette frequency, 24.0% (SE, 1.8), $p < 0.001$, Student's *t* test). No statistically significant reversal of rosetting was seen with LHR-A or LHR-D⁺ (Fig. 1B). The entire domain of LHR-C was not tested, however, we did test a recombinant protein comprising the first three SCRs of LHR-C (SCRs 15–17). This region includes the C3b binding site and is virtually identical (three-amino acid differences) to the first three SCRs of LHR-B (SCRs 8–10). The recombinant protein SCRs 15–17 also significantly reversed rosetting compared with the control culture (Fig. 1C, control mean rosette frequency, 60.1% (SE, 2.7); SCRs 15–17 (500 µg/ml) mean rosette frequency, 36% (SE, 5.6), $p < 0.01$ Student's *t* test; SCRs 15–17 (50 µg/ml) mean

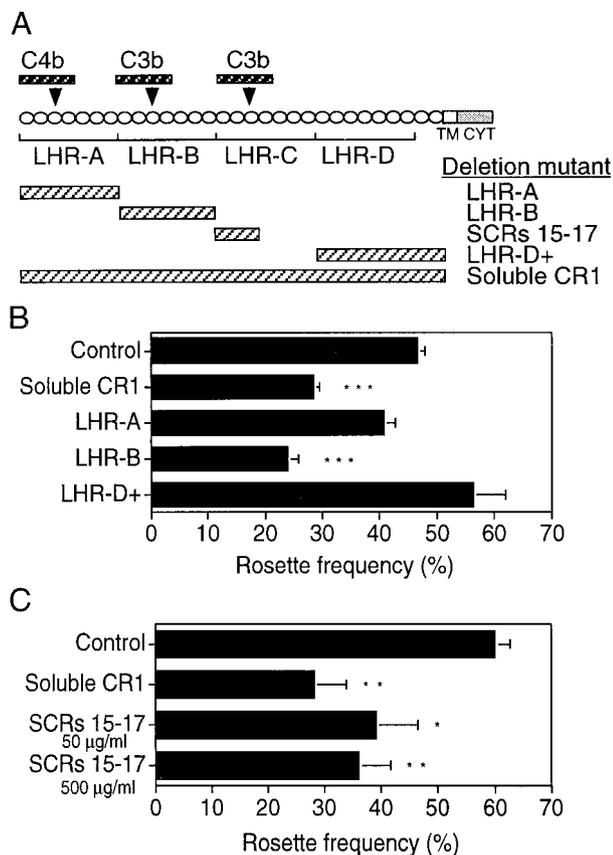


FIGURE 1. Reversal of *P. falciparum* rosetting by CR1 deletion mutants. *A*, A schematic diagram of the commonest allelic variant of CR1 showing the positions of the deletion mutants. Each SCR domain is represented by a circle, and the arrangement of SCRs in homologous groups of seven to form LHRs is shown. TM is the transmembrane region, CYT is the cytoplasmic tail, and the preferential binding sites for C3b and C4b are indicated by dark stippled boxes. The epitopes recognized by the CR1 mAb J3B11 (SCRs 3, 10, and 17) are indicated by arrowheads. *B*, Rosette reversal by 50 µg/ml CR1 deletion mutants and soluble CR1 in parasite clone R29. The control culture was incubated with culture supernatant from mock-transfected COS-7 cells. *C*, Rosette reversal by SCRs 15–17 (50 and 500 µg/ml) and soluble CR1 (50 µg/ml). The control was a culture with no added recombinant protein. The values given are means and SE of rosette frequency from four independent experiments in both *B* and *C*. Values that are statistically significantly different from the control value using Student's *t* test are asterisked (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

rosette frequency, 39.0% (SE, 7.5), $p < 0.05$, Student's *t* test). These data suggest that the region of CR1 that interacts with infected erythrocytes to form rosettes is in LHR-B and LHR-C and includes the first three SCRs of LHR-C (the C3b binding site). On a molar basis, the full-length soluble CR1 reversed rosettes more effectively than LHR-B and SCRs 15–17, presumably reflecting

the presence of multiple C3b binding sites in the full-length protein.

Rosette reversal by mAbs to CR1

Further elucidation of the region of CR1 required for *P. falciparum* rosetting came from studies of rosette reversal by mAbs to CR1. The epitopes recognized by the various mAbs have been mapped to particular SCRs within CR1 (Table I; data from Ref. 9). Most of the mAbs bind at several locations due to the repetitive nature of CR1. In most cases, it is not known whether the mAbs that group together have identical activities, because the fine specificity of their epitopes has not been determined; therefore, all mAbs in Table I were tested independently. Each Ab was tested at 1 and 10 µg/ml for its ability to reverse rosetting in clone R29 and compared with isotype control mAbs and control cultures with no added Ab. J3B11 was the only mAb to reverse rosetting (Table I), giving maximum reversal at 1 µg/ml (Fig. 2*A*, control mean rosette frequency, 55.5% (SE, 6.7), J3B11 (1 µg/ml) mean rosette frequency, 20.4% (SE, 4.6), $p < 0.01$, Student's *t* test). Higher concentrations of J3B11 (10 and 100 µg/ml) caused microscopic agglutination of erythrocytes in some experiments; therefore, rosette reversal at these concentrations was not studied further. Agglutination was not seen with any other Ab or reagent tested in this study. The epitope recognized by J3B11 has been mapped to SCRs 3, 10, and 17 (Fig. 1*A*), and this mAb is known to inhibit the rosetting of C3b-coated erythrocytes to CR1 on lymphocytes (14). Two other mAbs, 1B4 and 3D9, also map to the C3b binding site on CR1 (9) and block C3b-mediated rosetting (21). We considered the possibility that these mAbs may have been ineffective at reversing rosettes due to a lower affinity for CR1 than J3B11, or due to their epitopes being masked when infected erythrocytes are bound to CR1 in rosettes. We therefore conducted an alternative assay in which rosettes were mechanically disrupted in the presence of the mAbs to allow access of the Ab to CR1 and then assessed inhibition of the reformation of rosettes (the rosettes reform spontaneously after such mechanical disruption). It was found that mAbs 1B4 and 3D9 gave similar rates of inhibition to J3B11 when tested for their ability to inhibit rosette reformation after rosettes had been mechanically disrupted (Fig. 2*B*). The differences between the activities of these Abs may therefore reflect the precise epitope recognized by each mAb (the fine specificity of their epitopes is not known, and it is unknown whether these mAbs cross-inhibit each other) or may simply reflect differing affinities for CR1. In a single experiment, the other mAbs in Table I were also assessed for their ability to inhibit rosette reformation in clone R29. No inhibition was seen with any of the other mAbs.

Inhibition of rosette reformation by C3b dimers

Because the inhibition of the rosette reformation assay appeared to be more sensitive than the rosette reversal assay, we used the former to examine the ability of C3b dimers to inhibit rosette reformation in clone R29. Consistent with the other data, rosetting

Table I. Epitopes of CR1 mAbs^a and their effect on rosetting in parasite clone R29

mAb	Epitope (SCR no.)	Rosette Reversal ^b
1B4, 3D9, J3B11	3, 10, 17	Yes (J3B11)
J8B10, J3D3, 7G9, 9H3, YZ1	5–7, 12–14, 19–21	No
E11, HB8592	5–7, 12–14, 19–21, 26–28	No
1F11.G12	5–7, 12–14	No
3C6.D11, 6B1.H12	22–30	No

^a Data from Ref. 9.

^b mAbs were tested at 1 and 10 µg/ml for their ability to reverse rosetting (see text for details). Each mAb was tested in at least three independent experiments.

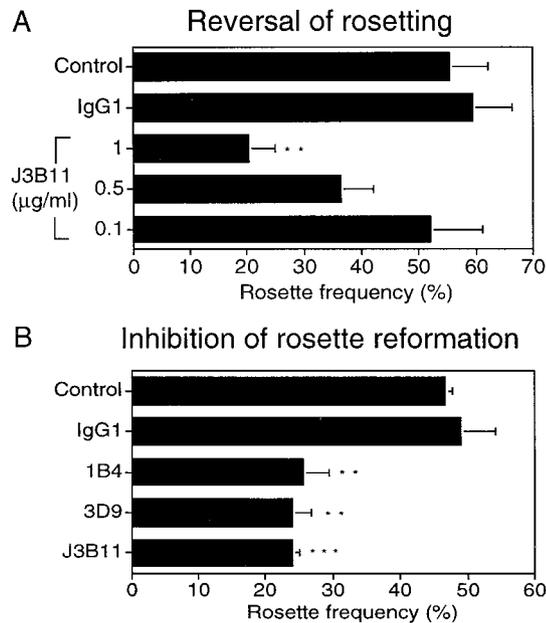


FIGURE 2. Reversal and inhibition of *P. falciparum* rosetting by CR1 mAbs. *A*, Rosette reversal by 1, 0.5, and 0.1 µg/ml mAb J3B11 in parasite clone R29. Values shown are mean rosette frequency and SE from four independent experiments. *B*, Inhibition of rosette reformation in clone R29 by 1 µg/ml mAbs 1B4, 3D9, and J3B11. Rosettes were mechanically disrupted and then allowed to reform in the presence of Ab. The values shown are the mean rosette frequency and SE from three independent experiments. For both *A* and *B*, control is a culture with no added Ab and IgG1 is an isotype control mAb at 1 µg/ml. Values that are statistically significantly different to the no Ab control value using Student's *t* test are asterisked (**, $p < 0.01$; ***, $p < 0.001$).

was inhibited by ~50% in the presence of 100 µg/ml (~300 nM) of C3b dimer (Fig. 3). Taken together, the results from the deletion mutants, the mAbs, and the C3b dimers all indicate that the region of CR1 required for *P. falciparum* rosetting maps to the C3b binding site in LHR-B and LHR-C, and particularly involves SCRs 10 and 17.

Requirement for C3b in rosetting

One interpretation of the above data is that rosetting might occur by C3b bridging between the infected erythrocyte and CR1. To examine this hypothesis, we grew parasites R29, TM180, and PAR⁺ in complete RPMI 1640 containing 10% C3-deficient se-

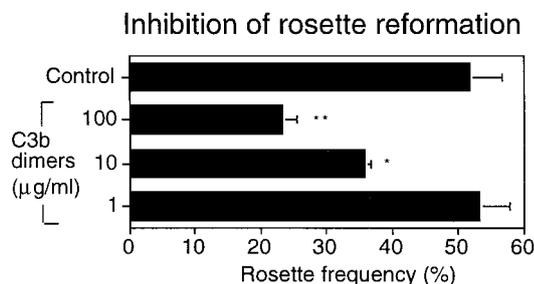


FIGURE 3. Inhibition of *P. falciparum* rosetting by C3b dimers. Rosettes were mechanically disrupted and then allowed to reform in the presence of 1, 10, and 100 µg/ml of C3b dimers. The values shown are the mean rosette frequency and SE from three independent experiments with clone R29. Control is a culture with no additive. Values that are statistically significantly different to the control value using Student's *t* test are asterisked (*, $p < 0.05$; **, $p < 0.01$).

rum and compared rosetting to control cultures in RPMI 1640 with 10% normal human serum. Parasites in C3-deficient serum invaded erythrocytes as efficiently as the control cultures (data not shown) and were morphologically normal. Rosetting in C3-deficient serum was the same as that seen in the control cultures (Table II), indicating that C3b is not required for *P. falciparum* rosetting.

The role of CR1 in rosetting in other laboratory strains and field isolates

The above experiments with parasite clone R29 show that approximately half to two-thirds of the rosettes are inhibited by CR1 mAbs and deletion mutants. Clearly other erythrocyte molecules may also contribute to rosetting in this parasite, and other candidate receptors that have been identified include CD36 (20), ABO blood group oligosaccharides (17), and heparan sulfate-like molecules (22). Serum factors such as Igs in normal human serum also play a role in rosetting in some parasite lines (23–25), possibly by stabilizing the interaction between the infected and uninfected erythrocytes (25). The extent to which these various rosetting mechanisms operate in different parasite isolates is not well understood. Therefore, we set out to study the role of CR1-mediated rosetting in a range of laboratory strains and natural parasite populations. We tested the ability of the CR1 mAb J3B11 to reverse rosettes in various isolates, and, in some experiments, we also studied the effect of mAb OKM5 that reverses CD36-mediated rosetting (20) and the effect of a rabbit polyclonal antihuman Ig reagent. In three of four laboratory strains (including R29), rosettes were reversed by J3B11, none were reversed by the CD36 mAb OKM5, and three of four were reversed by the anti-Ig Abs (Fig. 4A), including clone PAR⁺ which has been shown previously to be highly dependent on IgM for the formation of rosettes (23, 24). In 14 of 15 Kenyan field isolates, rosetting was reduced to <75% of the control value in the presence of J3B11, and in several of these isolates rosetting was virtually abolished (Fig. 4B). The antihuman IgA/IgG/IgM polyclonal Ab also reversed rosetting in many of these isolates, although to a lesser extent than J3B11 (Fig. 4B). No rosette reversal occurred with the CD36 mAb, confirming that CD36 only rarely plays a role in rosetting (26). In the Malawi isolates, in 5 of 10 isolates, rosettes were reversed by J3B11 (other Abs not tested) (Fig. 4B). These data show that CR1 is important in rosetting in the majority of field isolates and confirm that Igs also have a role to play in many isolates.

Discussion

In this report, we have further characterized the interaction between *P. falciparum*-infected erythrocytes and CR1 on uninfected erythrocytes that leads to the formation of rosettes. Taken together, the results from the deletion mutants, the mAbs, and the C3b dimers all indicate that the region of CR1 required for rosetting with parasite clone R29 maps to the C3b binding site of CR1 in LHR-B and LHR-C, and particularly involves SCRs 10 and 17. These results raise the interesting question of whether the activated complement component C3b could be involved in the formation of

Table II. Rosetting in C3-deficient human serum

Parasite	Normal Human Serum (%)	C3-Deficient Serum (%)
R29	73 ^a	70
TM180	61	61
PAR ⁺	44	50

^a Percentage of mature infected erythrocytes in rosettes. Values are from a single experiment.

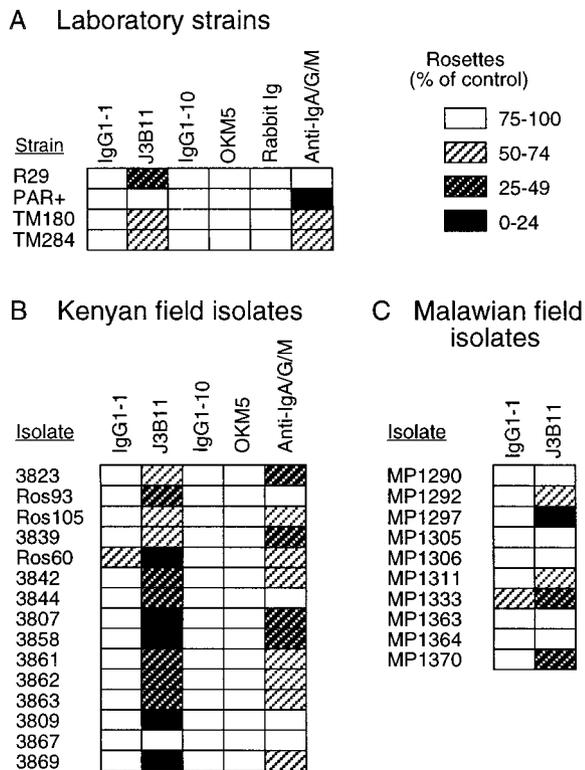


FIGURE 4. Reversal of *P. falciparum* rosetting by the CR1 mAb J3B11 in laboratory strains and field isolates. *A*, Rosette reversal by J3B11 (1 μ g/ml), OKM5 (anti-CD36 mAb, 10 μ g/ml), and antihuman IgA/IgG/IgM (rabbit polyclonal Ab, 1/50 dilution) in parasite clones R29 and PAR⁺ and lines TM180 and TM284. Rosetting is shown as the percentage of a control culture with no added Ab, and the values represent the means of at least three experiments. IgG1-1 is an isotype control at 1 μ g/ml, IgG1-10 is an isotype control at 10 μ g/ml, and rabbit Ig is nonimmune rabbit Ig at 1/100 dilution. *B*, Rosette reversal by J3B11 (1 μ g/ml), OKM5 (10 μ g/ml), antihuman IgA/IgG/IgM (1/50 dilution), and controls (as above) in field isolates from malaria patients in Kilifi, Kenya. Values are from a single experiment, because the majority of field isolates will not grow in continuous culture to allow repeated experiments. *C*, Rosette reversal by J3B11 and IgG1 isotype control at 1 μ g/ml in field isolates from malaria patients in Blantyre, Malawi. Values are from a single experiment.

P. falciparum rosettes. It has been shown previously that the parasite-rosetting ligand in the R29 clone is a member of the PfEMP1 family (8), a parasite protein that is expressed on the surface of infected erythrocytes. Heterologous expression in COS-7 cells of the N-terminal domain of the PfEMP1 variant from clone R29 was shown to mediate binding to erythrocytes in a CR1-dependent fashion (8). The above mapping data suggest either that by coincidence PfEMP1 binds to the same region of CR1 as C3b, or, alternatively, that the binding of infected erythrocytes to CR1 occurs via C3b "bridging" between PfEMP1 and CR1. Two papers in the literature suggest that C3b is found on the surface of infected erythrocytes grown *in vitro* in nonimmune human serum (27, 28), although there is nothing in these reports to suggest that this phenomenon is linked to rosetting. Many pathogenic microorganisms are known to manipulate the host's complement system for their own ends and to use the host's complement receptors to facilitate adhesion and invasion of tissues (reviewed in Refs. 29 and 30). For example, *Leishmania major* (31, 32), *Legionella pneumophila* (33), *Mycobacterium leprae* (34), and *Mycobacterium tuberculosis* (35) all activate complement and become coated with C3b or its derivatives, and then enter host macrophages via CR1 or CR3. Other pathogens express surface ligands that bind directly to com-

plement control proteins, such as the Dr-like Ags of *Escherichia coli* that adhere to decay-accelerating factor (36), and measles virus hemagglutinin that binds to CD46 (37). To determine which of these strategies has been adopted by *P. falciparum*, we studied the requirement for C3b in rosetting by growing parasites in medium containing C3-deficient human serum. In three parasite lines, rosetting in the C3-deficient serum was indistinguishable from that in normal serum, indicating that C3b is not required for the formation of rosettes and suggesting that PfEMP1 binds directly to CR1 and acts as a C3b mimetic. There is no apparent sequence homology between PfEMP1 and C3b; however, PfEMP1 does contain regions with a preponderance of acidic amino acids, which have been shown to be crucial in the interaction between C3b and CR1 (38). Additional experiments are underway to localize more precisely the region of PfEMP1 that interacts with CR1 and to determine whether such a region is well-conserved among different rosetting parasite isolates.

Our results also confirm the reports of others (23–25) that Igs found in normal human serum are required for rosetting, as rosetting was inhibited by polyclonal Abs to human Igs in 3 of 4 laboratory strains and 11 of 15 field isolates. It is unclear whether the requirement for Ig is linked in any way to the CR1-dependent rosetting mechanism. One hypothesis that would combine the two phenomena is that binding of a natural Ab to the surface of infected erythrocytes (possibly to PfEMP1) leads to complement activation and the deposition of C3b on the infected erythrocyte surface, which then contributes to rosetting by binding to CR1. However, as described above, in this report we have excluded a role for C3b in rosetting, and current data also indicate that Ig of defined specificity is not involved in the rosette-enhancing effect. In one report, two different monoclonal IgM preparations derived from patients with Waldenström's macroglobulinemia were able to support rosetting (23), suggesting that the effect of Ig is not due to recognition of a particular Ag, but occurs through a nonspecific aggregating effect.

Despite the apparent heterogeneity in rosetting mechanisms described to date (8, 17, 20, 22, 23–25), the data described in this report indicate a CR1-dependent mechanism of rosetting that occurs commonly and could, therefore, be a target for rosette-reversing therapies to treat severe malaria. The mapping studies, however, indicate that the region of CR1 required for rosetting includes the C3b binding site, and the only effective rosette-reversing mAb, J3B11, is known to inhibit binding of C3b to CR1 (14). Therefore, although interventions aimed at inhibiting CR1-dependent rosetting may be feasible, they could have the dual effect of interfering with other CR1 functions such as phagocytosis and clearance of immune complexes (1). Due to the intricate host-parasite interactions seen in malaria infection, such intervention could have harmful as well as beneficial effects and clearly should not be embarked upon without considerable forethought and further research.

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