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Processing of C3b-Opsonized Immune Complexes Bound to Non-Complement Receptor 1 (CR1) Sites on Red Cells: Phagocytosis, Transfer, and Associations with CR1

Maria L. Craig,* John N. Waitumbi,† and Ronald P. Taylor2*

Severe anemia is a lethal complication of Plasmodium falciparum malaria, particularly in children. Recent studies in children with severe P. falciparum anemia have demonstrated elevated levels of E-bound Abs, reduced E-associated complement receptor 1 (CR1) and decay-accelerating factor (DAF), and pronounced splenic enlargement, suggesting a mechanism for E loss involving Abs, complement, and phagocytosis. Motivated by these reports, we have developed an in vitro model in which human E with Abs and complement bound to CR1, DAF, or glycophorin A are incubated with model human macrophages (the THP-1 cell line). Previous work has demonstrated that immune complex (IC) substrates bound to E CR1, either by an Ab or via C3b, are transferred to macrophages with loss of CR1. In this study, we report that IC bound to DAF or glycophorin A by an Ab linkage are also transferred to macrophages. DAF is lost from the E during the transfer of DAF-bound IC, but the transfer of CR1-bound IC does not lead to a significant loss of DAF. Using glycophorin A-bound IC, we observe competition between transfer of IC and phagocytosis of the E: a fraction (≤15%) of the E was phagocytosed, while the remaining E were stripped of IC. We also examined the organization of CR1 and DAF in the presence of E-bound Ab/complement. We find that CR1, but not DAF, colocalizes with IgM mAb-C3b and IC-C3b substrates attached to glycophorin A. We observe that the binding of the IgM mAb-C3b to glycophorin A induces a novel unclustering of CR1. The Journal of Immunology, 2005, 174: 3059–3066.

M alaria is caused by a protozoan parasite, Plasmodium falciparum, which in the pathogenic stage of its life cycle forms merozoites and invades E. Severe anemia in children <5 years of age is one of the most serious complications of P. falciparum malaria, and this and other complications cause the death of >1 million children each year in sub-Saharan Africa (1). Only a small fraction of a child’s E becomes infected (1, 2), and therefore, direct lysis of the infected E cannot explain the severe anemia (3). The presence, in the circulation of malaria-infected animals and patients (4–9), of anti-malaria Abs, malarial Ags, immune complexes (IC), and complement degradation products, suggests that E loss may occur by a mechanism involving the interaction of Abs, IC, and complement with the E.

Binding of these substrates to E in vivo may lead to one of several possible fates for the E: hemolysis by complement attack (10), erythrocytotoxicity by fixed tissue macrophages in the liver and spleen (10–15), or removal of E-bound Abs or IC by transfer to macrophages (16, 17). Under normal circumstances, E are protected from hemolysis by the presence of complement control proteins, including complement receptor 1 (CR1), decay-accelerating factor (DAF or CD55), and CD59 (membrane inhibitor of reactive lysis) (18). CR1, organized in clusters on primate E (19–21), appears to be a privileged site in comparison with other E proteins such as glycophorin A, the Rho (D) Ag, or band 3, in that the binding of IgG and/or IC to CR1 leads to little in vitro erythropagocytosis (21, 22). Similarly, in vivo, binding of IC to E via CR1 does not induce E destruction (23, 24), while formation of IC at other sites promotes E lysis and/or clearance (11). In the first case, E are spared because IC bound to CR1, either via the activated large cleavage fragment of the third component of complement (C3b) or through the action of an anti-CR1 Ab, are taken up by macrophages in the liver and spleen in a process known as the transfer reaction (25). Studies in both in vitro and in vivo models delineate a mechanism for this transfer in which engagement of CR1-bound IC by macrophage Fc receptors leads to cleavage of CR1, resulting in loss of CR1 and the associated IC from the E (22, 25–28). Thus, the transfer reaction is likely to explain, at least in part, the reduced CR1 levels in patients with a variety of diseases involving circulating IC (29), particularly autoimmune diseases (29, 30) and AIDS (31). In the second case, E that have Abs and complement bound to other sites are removed and destroyed by fixed tissue macrophages (10–15).

Recently, studies in children with severe malarial anemia have suggested that E-bound Abs or IC may play important roles in the pathogenesis of malarial anemia (1, 9). As in autoimmune diseases and in AIDS, CR1 levels on the E of these children are reduced; in addition, E levels of a second complement-regulatory protein, DAF, are also reduced. Other evidence suggestive of involvement of E-bound Abs or IC in the pathogenesis of malarial anemia includes increased levels of circulating IC and E-bound IgG, increased susceptibility of the E to in vitro phagocytosis, and splenic enlargement (1, 9). Abs to malarial proteins displayed on the surface of infected E have been identified (32, 33), but the mode of Ab binding to uninfected E is not well understood. Glycophorin A, B, and C, being among the most abundant E surface proteins, are
some of the receptors used by *P. falciparum* during entry into E (34–37). These intriguing findings have motivated us to initiate in vitro studies aimed at understanding what happens when Abs and complement are bound at non-CR1 sites on the E, in particular DAF and glycoporphin A.

Our study has yielded several novel results regarding the transfer reaction and the organization of CR1 on the E surface. Notably, we find that DAF- or glycoporphin A-bound IC (Fig. 1A) consisting of multiple Abs, with or without complement, are transferred from E to model macrophages. The transfer of IC bound to DAF leads to the loss of DAF from the E, and this loss of DAF appears to occur independently of the loss of CR1. When the IC are bound to glycoporphin A, we observe, in agreement with Reimagel et al. (21), that a fraction of the E is phagocytosed; however, we find that the E that escape phagocytosis are almost completely stripped of IC by the macrophages. Fluorescence microscopy of E with IC bound to glycoporphin A shows that CR1, but not DAF, colocalizes with the IC when complement is activated and C3b deposits on the E-bound IC during opsonization. Moreover, when IgM Abs are used to target glycoporphin A and promote C3b deposition, we observe an unusual binding pattern characterized by rearrangement and unclustering of CR1.

### Materials and Methods

#### Antibodies

The anti-CR1 mAbs (38) 1B4, HB8592, and 9H3, and the anti-C3b Abs 7C12, 9F9, and 10H5 (39, 40) were produced at the University of Virginia Lymphocyte Culture Center and were labeled with Alexa (Al) 488 dye (kit from Molecular Probes) or EZ-Link N-hydroxysuccimide-long chain-biotin (Pierce). Anti-C3b mAbs IA10 (biotinylated; BD Pharmingen) and BRIC216 (BioSource International) have been described previously (41). Mouse IgM Ab to glycoporphin A (A63-B/C2) and PE-labeled mouse IgG1 mAb to leukocyte common Ag (CD45, mAb HI30) were obtained from Ancell and Caltag Laboratories, respectively. Commercially available secondary Abs were as follows: Al488- and Al594-labeled rabbit anti-mouse IgG, Al594-labeled goat anti-rabbit IgG, and Al488- and Al594-labeled streptavidin were purchased from Molecular Probes; unlabeled and Cy5- and Texas Red-labeled rabbit IgG anti-mouse IgM were purchased from Jackson Immunoresearch Laboratories; and unlabeled rabbit IgG anti-mouse IgM was also obtained from Cortex Pharmaceuticals.

#### Cells

E were obtained from blood samples of normal human donors and were washed thoroughly with BSA-PBS (1% BSA in PBS) before use. THP-1 cells were cultured and treated with all-trans retinoic acid, as described previously (17).

#### Cell labeling

E (1 × 10^7 cells/ml) were mixed with 6 μM PKH67 dye in Diluent C (both from Sigma-Aldrich) for 5 min at room temperature. An equal volume of BSA-PBS was added to stop the reaction and, after centrifugation and removal of the supernatant, E were washed twice more with BSA-PBS. Alternatively, E (5 × 10^6 cells/ml) were combined with 24 μM 5-chloromethylfluorescein diacetate (CMFDA; Molecular Probes) in PBS for 15 min at 37°C, and, after centrifugation and removal of the supernatant, were diluted to 2 × 10^6 cells/ml in PBS, held at 37°C in cold PBS, and washed thoroughly with BSA-PBS.

#### Binding of IC and complement to DAF and CR1

E (5 × 10^6 cells/ml) were incubated for 10 min at 37°C with anti-DAF (50 μg/ml BRIC 216 or a 1.5-fold dilution of biotinylated IA10) or with anti-CR1 (90 μg/ml 9H3 or 1B4) mAbs, washed, and then further opsonized for 10 min at 37°C with 0.1 mg/ml unlabeled or Al633-labeled rabbit anti-mouse IgG. E were washed again, and, in some cases, cell pellets were then suspended at 2 × 10^6 cells/ml in neat human autologous serum, held for 10 min at 37°C, and thoroughly washed.

#### Binding of IgM Abs, IC, and complement to glycoporphin A

E (1 × 10^6 cells/ml) were mixed with subsaturating amounts (4.5 μg/ml) of either unlabeled or an Al647-labeled IgM anti-glycoporphin A mAb for 10 min at 37°C, washed, and then incubated for 10 min at 37°C with 9 μg/ml unlabeled, Cy5-labeled, or Texas Red-labeled rabbit IgG anti-mouse IgM. E were then allowed to activate complement by treatment with serum, as above. Control samples were prepared in which serum contained 10 nM EDTA to block complement activation.

#### Transfer/phagocytosis reactions

E (1 × 10^6 cells/ml in BSA-PBS) and THP-1 cells (2 × 10^7 cells/ml in culture medium, but lacking PBS and gentamicin) were combined at varying ratios, mixed, and pelleted by centrifugation (1 min, 2000 × g, 4°C). After incubating at 37°C, without shaking, for 30 min or 1 h, cells were respectively analyzed by flow cytometry or fluorescence microscopy. In some flow cytometry experiments (Fig. 2), E were separated from THP-1 cells by layering over 60% Percoll (Amersham Biosciences) in PBS, followed by centrifugation (4°C, 1250 × g, 20 min). In other experiments (Fig. 3), the cell pellets were resuspended in BSA-PBS to a THP-1 concentration of 1 × 10^7 cells/ml and, to selectively stain the THP-1 cells, PE anti-CD45 mAb was bound (1/20 final dilution) by incubating for 10 min at 37°C. In certain cases (Fig. 3D), E that had not been internalized were removed by hypotonic lysis in 1.5 ml of 0.1% (w/v) NaCl for 1 min (21). For fluorescence microscopy (Fig. 4), the reactions were stopped by placing the tubes on ice and resuspending, using repetitive pipetting, in 1 ml of cold BSA-PBS containing 2 mg/ml rabbit or human IgG. As a negative control (Fig. 4A), E and THP-1 cells were added directly to 1 ml of ice-cold BSA-PBS containing 2 mg/ml rabbit or human IgG. The cells were pelleted (2 min, 2000 × g, 4°C) and fixed by suspending in 500 μl of 1% paraformaldehyde in PBS.

#### Probing of native and opsonized E with fluorescently labeled Abs and streptavidin

E at ~5 × 10^6 cells/ml were separately incubated with one or more probes at the following concentrations for 10 min at 37°C and then washed with BSA-PBS: 70 μg/ml Al488-rabbit anti-mouse IgG; a 2.5-fold dilution of biotinylated IA10 (anti-DAF); 50 μg/ml Al488-streptavidin; 40 μg/ml biotinylated 9H3 (anti-CR1); and 50 μg/ml Al488-7C12 (anti-C3b). For the fluorescence microscopy studies (Figs. 5 and 6, below) of IC/receptor organization, E (9 × 10^6 cells/ml) were incubated for 10 min at 37°C or 1 h at 4°C with the following IgG mAb probes, all at a concentration of 9 μg/ml, unless noted (targeted molecule in parentheses): Al488-7C12 (C3b); Al488-9F9 (C3b); Al488-10H5 (C3b); biotinylated 9H3 (CR1); biotinylated HB8592 (CR1); biotinylated IA10 (50-fold dilution, DAF); and Al594-goat anti-rabbit IgG (50 μg/ml, polyclonal). Biotinylated IA10 (DAF) and Al594-streptavidin, respectively, were at a 50-fold dilution and 50 μg/ml. For all probes except anti-mouse IgG, excessive mouse IgG (~2 mg/ml) was added to prevent binding of the probes to anti-mouse IgGl/IIg already incorporated into IC on the E.

#### Fluorescence microscopy

Cells (10^4-10^5 per slide) were placed on poly(t-lysine)-coated slides (Erie Scientific) either by centrifugation at 1000 rpm for 3 min in a Cytospin 4 centrifuge (Thermo Shandon) or by adherence of 10 μl of a suspension. Prolong antifade reagent (Molecular Probes) was used to maintain fluorescent signals. Cells were covered with glass coverslips (No. 1, 18 × 18 mm) and sealed with nail varnish, and slides were examined using a BX40 fluorescent microscope (Olympus), equipped with a Magnafire digital camera.

#### Flow cytometry

E, THP-1 cells, and E/THP-1 mixtures were analyzed using CellQuest software on a FACScalibur flow cytometer (BD Biosciences). Fluorescence intensities were converted to molecules of equivalent soluble fluorochrome using calibrated standard beads (Spherotech).

To determine the levels of the anti-glycoporphin A substrates (FL4 positive) bound to E and THP-1 cells in the transfer/phagocytosis experiments, we separated signals on E from those on THP-1 cells using a gating scheme to distinguish THP-1 cells with internalized E from those with intact E. THP-1 cells were distinguished from intact E on the basis of their lower forward and side scatter. Inclusion and exclusion gates were used simultaneously to insulate that THP-1 cells that had internalized E did not contribute to the FL4 signal of the E. However, our gating scheme did not exclude THP-1 cells with internalized E from contributing to the total signal of the THP-1 cells.
Results
DAF-bound IC on E are transferred to THP-1 model macrophages

To examine possible mechanisms for the reduction in E-associated CR1 and DAF in malaria, we created model IC (Fig. 1) bound to either CR1 or DAF by first binding anti-CR1 or anti-DAF mAbs to E and subsequently adding Al633-labeled rabbit anti-mouse IgG. In some cases, these IC were then allowed to activate complement to induce C3b deposition. We incubated E containing these IC either with retinoic acid-treated THP-1 cells or in buffer alone, and, after separation of the E from the macrophages, used Al488-labeled Abs to determine levels of mouse IgG, CR1, or DAF on the E.

Strikingly, the processing of DAF IC closely resembles processing of CR1 IC (Fig. 2). In particular, two key results are evident: 1) the DAF-bound IC, like the CR1-bound IC, are removed from the E in the presence of the macrophages, and 2) this loss of DAF- and CR1-bound IC from the E is accompanied by respective loss of the IC-bearing receptor (Fig. 2A). After binding of C3b, we observed similar trends, except that the loss of DAF from the E was not as pronounced (Fig. 2B). In addition, the majority of the C3b was also lost from the E (Fig. 2B).

Glycophorin A-bound IC are transferred to THP-1 model macrophages

We also examined the handling of Abs and IC bound at an additional E site, glycophorin A. We chose this site for several reasons: first, previous studies of the phagocytosis of E with glycophorin A-bound IC created a framework for this work (21); second, glycophorin A is abundant on the E; and, third, glycophorin A has been implicated as a site of P. falciparum entry into E (36). The abundance of glycophorin A on E necessitated the use of subsaturating amounts of anti-glycophorin A mAb to minimize cross-linking of the E. We compared the handling of four different types of substrates bound to glycophorin A, as illustrated in the Fig. 1 schematic (figure derived from Ref. 42). These substrates were prepared with an IgM anti-glycophorin A mAb, rabbit IgG anti-mouse IgM Abs, and serum as a complement source (for C3b opsonization). Levels of binding of the IgM anti-glycophorin A mAb to E were similar for three of the four substrates; however, binding under similar conditions was 80% lower for the IC-C3b substrate, possibly due to complement-induced disruption of the IC (43).

To examine both phagocytosis and transfer simultaneously, we designed a three-color system in which E, THP-1 cells, and substrates were labeled with, consecutively, PKH67 (green), PE anti-CD45, and either Al647-IgM anti-glycophorin A mAb or Cy5-rabbit anti-mouse IgM. The phagocytosis of E by THP-1 cells produced a population of green/PE double-positive cells that could be measured by flow cytometry, and therefore, we measured phagocytosis by enumerating these green/PE double-positive cells after an E lysis step that selectively removed E that had not been internalized. Transfer was evaluated using flow cytometry to follow loss from the E and uptake by the THP-1 cells of the Al647-IgM anti-glycophorin A or Cy5-rabbit anti-mouse IgM.

We found that both phagocytosis and transfer occurred, with phagocytosis of up to 15% of E that escaped phagocytosis were stripped of most of the bound IC and IC-C3b (Fig. 3A), and uptake of both substrates by the THP-1 cells was apparent (Fig. 3B). For reasons we do not understand, more loss from the E of the non-IC mAb and mAb-C3b substrates was observed in a mock control (lacking THP-1 cells) than in the presence of THP-1 cells (data not shown), and there was little uptake of these two substrates by the THP-1 cells (Fig. 3B).
We determined the percentage of the THP-1 cells internalizing E (percentage of phagocytosis) based on the ratio of green/PE double-positive cells to total PE-positive cells (Fig. 3C), and these values are reported in Fig. 3D. The IC substrate consistently induced the most phagocytosis, with the IgM mAb-C3b and IC-C3b substrates also inducing more phagocytosis than the IgM mAbs alone (Fig. 3D).

Fluorescence microscopy studies further confirmed the dual transfer/phagocytosis phenomenon. IC labeled with Al647 anti-glycophorin A (red) were bound to E labeled with CMFDA, a green tracking dye. The E were then used in the transfer/phagocytosis reaction, as described above. Before the reaction (Fig. 4A), the IC were observed as clusters of red fluorescence on the green-dyed E. After the reaction (Fig. 4B), the E were devoid of the IC-associated red color, which had been transferred to the THP-1 cells. After an E lysis step, instances of phagocytosis were observed (Fig. 4C). Fig. 4C clearly demonstrates the dual transfer/phagocytosis phenomenon, showing an E that has been phagocytosed by a THP-1 cell (arrow) adjacent to a THP-1 cell that has taken up IC in the transfer reaction (asterisk).

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FIGURE 5. CR1 organization on E reflects the positioning of glycophorin A-bound C3b. E with substrates (labels to the left of the figure) bound to glycoporphin A were probed at 4°C for CR1 (red) using a mixture of biotinylated mAbs (9H3 and HB8592) and Al594-SA and for C3b (green) using a mixture of Al488-labeled mAbs (7C12, 9F9, and 10H5). When an IgM mAb is bound (top row), CR1 retains its normal clustered, punctate distribution. However, when the IgM-coated E are incubated with serum to deposit C3b (second row), CR1 reorganizes, taking on a distribution like that of the C3b. When the cells are opsonized with IC and C3b (third row), CR1 and C3b colocalize in punctate clusters. Enlargement (fourth row) of an area from the third row clearly demonstrates the colocalization (note yellow colors in zoom overlay) between CR1 and C3b. Results are representative of two to four similar experiments.

FIGURE 6. DAF organization on E is independent of the positioning of glycophorin A-bound C3b. Experimental procedures were the same as Fig. 5, except that E were probed for DAF (red), instead of CR1, using biotinylated mAbs (IA10) and Al594-SA. DAF is organized in punctate clusters when the IgM mAb is bound (top row). Unlike CR1, DAF does not reorganize when C3b is deposited on the IgM-coated E (second row). Furthermore, DAF clusters do not colocalize with C3b clusters when the substrate is IC-C3b (third and fourth rows). Note the preponderance of independent green and red spots. Results are representative of two to four similar experiments.
CR1, but not DAF, colocalizes with mAb-C3b and IC-C3b bound to glycophorin A on E

To determine whether CR1 and DAF interact with Ab/complement substrates bound to non-CR1, non-DAF sites on E, we used fluorescence microscopy to examine the same four anti-glycophorin A substrates studied above. In this experiment, unlabeled substrates were bound to E and followed by fluorescently labeled mAb probes specific for CR1, DAF, or C3b (Figs. 5 and 6). Interestingly, we found that CR1 signals increased when the complement-opsonized substrates, but not the noncomplement-opsonized substrates, bound to E (2- to 4-fold for mAb-C3b and 2- to 3-fold for IC-C3b based on molecules of equivalent soluble fluorochrome values; data not shown). Results of probing for 10 min at 37°C agreed well with results of probing for 1 h at 4°C, indicating that reorganization of molecules on the E surface was not due to probe binding. In addition, we confirmed that C3b deposited on the E colocalized with the IgM anti-glycophorin A mAb (data not shown).

We addressed the issue of association between CR1 or DAF and the glycophorin A-bound C3b. CR1 is organized in clusters on E (19-21), but glycophorin A is distributed more evenly over the E surface (21). The IgM anti-glycophorin A mAb used in these studies binds evenly across the entire E surface (data not shown) without altering the clustered organization of CR1 (Fig. 5, top row). In contrast, binding of C3b to E opsonized with this mAb induces loss of CR1 clustering (Fig. 5, second row). Conversion of these mAbs to IC by the addition of IgG anti-IgM mAbs induces a clustering and condensation of the mAbs (Fig. 4A), and, when C3b is deposited on these IC clusters, we observe substantial colocalization of CR1 clusters with the deposited C3b clusters (Fig. 5, third and fourth rows). A comparable experiment using the anti-glycophorin A mAb probes. Glycophorin A, however, colocalized with the IgM anti-glycophorin A mAb (data not shown).

We tested two hypotheses that could explain reduced levels of E DAF in children with malarial anemia: 1) DAF-bound IC (± complement) may be transferred to macrophages in a process resembling the CR1 transfer reaction and therefore involving loss of DAF from E; and 2) an interaction between CR1 (or CR1-bound C3b) and DAF may promote cotransfer of DAF and CR1. Although we could find no reports of anti-DAF Abs in malaria, our results (Fig. 2A) are consistent with hypothesis 1, showing that binding of IC to DAF leads to loss of IC and DAF from E. Thus, we speculate that Abs may bind to DAF in malaria, either directly or perhaps by way of DAF-bound *P. falciparum* proteins. In fact, several noncomplement proteins can bind to DAF during invasion by other pathogens (47). We found little evidence to support hypothesis 2. In the absence of C3b, CR1 or DAF loss was restricted to the IC-bound receptor (Fig. 2A). However, in the presence of C3b, a small loss of CR1 or DAF was observed when IC were bound, respectively, to DAF or CR1 (Fig. 2B), and thus, we cannot exclude the second hypothesis on the basis of these data. Finally, C3b binding to the receptor-specific IC leads to less DAF loss (from 49 to 32%; Fig. 2), and this might be due to partial blockade of the Fc regions of the anti-DAF mAb by C3b (28).

**Discussion**

**General approach**

Modifications of the E surface, including Ab binding, C3 fragment deposition, and reductions in CR1 and DAF, have been observed in malaria-infected children with severe anemia (1, 7, 9). When taken in combination with reports of malarial proteins on the E surface (44) and of low parasitemia in monkey models of severe malarial anemia (2), this evidence suggests that Abs and complement may bind to malarial proteins adhered to the surface of both infected and uninfected E, possibly causing loss of CR1 and DAF from E, and, ultimately, inducing E destruction. With the goal of understanding possible mechanisms for E loss, we developed an in vitro model in which Abs and complement are bound to select E sites. Our investigations reveal novel insights into the E-macrophage interactions and E surface rearrangements resulting from binding of complex substrates to E.

**Transfer of IC substrates from E to macrophages**

C3b-opsonized IC bound to E CR1 and B cell CR2 are transferred to macrophages in a process known as the transfer reaction. This process does not require complement, but occurs by a mechanism in which macrophage FcyR bind IC-associated Fc regions to form E (or B cell)-macrophage linkages that are released by the removal of CR1 (CR2) and bound IC from the cells, possibly by proteolytic cleavage of the receptors (17, 26, 28, 45). Our investigation reveals that IC bound at two other E sites, DAF (Fig. 2), which is structurally similar to CR1 and CR2 (18), and glycophorin A (Fig. 3, A and B; Fig. 4, A and B), which is structurally different (46), are also transferred to macrophages. Transfer can occur in the absence of complement (Fig. 2A), suggesting an FcγR-based mechanism like that described for CR1 and CR2. The DAF transfer reaction further resembles transfer of CR1 and CR2 because DAF is lost from E (Fig. 2) when DAF-bound mAb are removed. We were unable to investigate glycophorin A loss due to its abundance on E; use of saturating amounts of anti-glycophorin A caused E cross-linking, and we could not measure a reduction in glycophorin A when less Ab was used. Thus, we cannot address all details of the mechanism of release of glycophorin A-bound IC from E.

We report that the organization on the E surface of CR1, but not of DAF, can change in response to binding of C3b-opsonized Abs and IC to a non-CR1 site, glycophorin A. E CR1 is normally organized in clusters (19-21), characterized by a punctate fluorescence when visualized using Ab probes. Glycophorin A, however, is distributed more evenly over the E surface (21). We demonstrate...
in this study that C3b opsonization of IgM mAbs bound to glycophorin A induces marked changes in CR1 organization: CR1 becomes unclustered, taking on the distribution of IgM antibody binding. CR1 clusters are apparently weaker than those between CR1 and membrane proteins) of Fas-associated phosphatase-1 may contribute to CR1 clustering on E. In any case, interactions that maintain CR1 clusters are apparently weaker than those between CR1 and multimeric C3b, because interaction with the IC-C3b promotes disruption of the clusters.

We found no evidence for DAF colocalization with the complex CR1-C3b-mAb or CR1-C3b-IC (Fig. 6). This is consistent with the lack of cooperation between CR1 and DAF in the transfer reactions (Fig. 2B), and both results are perhaps expected because DAF binds to C3 convertases rather than to C3b. Thus, in this study, we cannot provide a mechanism for DAF loss in the children with severe malarial anemia.

Based on observations in children with severe P. falciparum anemia, we have developed an in vitro model to study IC- and complement-based mechanisms of E destruction. We have demonstrated that IC substances bound to non-CR1 sites on E, specifically DAF and glycophorin A, are transferred from E to macrophages, and that DAF is lost from E during this transfer process. In addition, we have found that binding of these substances to glycoporphin A induces either transfer of these substances to macrophages or phagocytosis of E by macrophages. Finally, we have observed that CR1 colocalizes with C3b on the E surface, even becoming unclustered when C3b is uniformly distributed over the E surface. These studies may provide the foundation for analysis of blood and E from children at diverse phases of malaria infections.

Disclosures

The authors have no financial conflict of interest.

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