

MEMBRANE-BOUND C4b INTERACTS ENDOGENOUSLY WITH COMPLEMENT RECEPTOR CR1 OF HUMAN RED CELLS

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Amplification of the complement cascade on cell surfaces is mediated by the membrane-bound C3 convertases of the classical (C4b2a) and alternative (C3bBb) pathways. These enzymes are assembled after the nonspecific covalent binding of nascent C4b or C3b to acceptor molecules on the cell membranes. These polypeptides can bind not only to foreign substrates, but also to the surface of autologous cells in the vicinity of the site of complement activation.

Until a few years ago, the mechanisms of control of the potentially harmful C3b and C4b that bind to autologous cells were obscure. Recent evidence (1-5) strongly suggests that the assembly of C3-convertases on these complement fragments is inhibited by specific membrane molecules. One of them is decay-accelerating factor (DAF),¹ a glycoprotein of ~75,000 M_r , found on all blood cells, endothelial, and epithelial cells (6-10). DAF inhibits the assembly of C3 convertases on autologous cells by binding to C4b or C3b, interfering with the association of these complement fragments with C2 or factor B (4, 11). However, DAFs action is reversible and does not alter the structure of C4b or C3b. A second inhibitor, complement receptor type 1 (CR1), binds to C4b and C3b, serving as a cofactor for the cleavage and irreversible inactivation of the complement fragments by the serum enzyme C3b/C4b inactivator (I). CR1 is a glycoprotein of ~200,000 M_r found on human erythrocytes, neutrophils, monocytes, B (and a few T) lymphocytes, and glomerular podocytes (reviewed in 12 and 13).

Although in theory CR1 could interact with clustered C3b/C4b deposited either on the same cell membrane (endogenous activity), or on neighboring cells (exogenous activity), only the latter has been well documented. The purpose of the experiments described in this paper was to determine whether CR1 has endogenous activity. We reasoned that if CR1 can move in the plane of the cell membrane and recognize the covalently attached C4b ligand, we should be able to detect this interaction by means of bifunctional crosslinking reagents. More-

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¹ *Abbreviations used in this paper:* DAF, decay-accelerating factor; DGVB²⁺, dextrose, gelatin, and isotonic veronal buffer with divalent cations; DSP, dithio-bis(succinimidyl propionate); HE, human erythrocyte; HEA, antibody-sensitized HE; I, C3b/C4b inactivator; SE, sheep erythrocyte; SEA, antibody-sensitized SE.

over, the formation of these CR1/C4b complexes within the cell membrane might have an inhibitory effect on the exogenous cofactor activity of CR1.

Materials and Methods

Buffers, Antibodies, Complement Components, and Red Cell Hemolytic Intermediates. We used isotonic veronal buffer containing 2.47 mM sodium veronal buffer (pH 7.3), 72.7 mM NaCl, 2.5% dextrose, 0.1% gelatin, 0.15 mM CaCl₂, and 0.5 mM MgCl₂ (DGVB²⁺); and we brought PBS to pH 8.3 with 0.1 N NaOH. mAbs to human CR1 (57F, 57H, and 44D) (14); to DAF (IA10, I1H⁶ and VIIIA7) (8); and to a protein from a malaria parasite (3D11, [15]); guinea pig C1 (16); human C4 (17), C2 (18), and C3 (17); factor I (19); and CR1 (20) were prepared as described. Rabbit anti-Forssman hemolysin was from Gibco Laboratories (Grand Island, NY) and affinity-purified goat IgG anti-mouse IgG was bought from Cappel Laboratories, (Cochranville, PA). Rabbit IgM antibodies to human erythrocytes (HE) were a gift of Dr. Eric Brown (Washington University, St. Louis, MO). IgG fractions from rabbit anti-C4 antiserum and normal rabbit serum were prepared by DEAE-cellulose chromatography.

C3, C4, and goat IgG anti-mouse IgG were radiolabeled with ¹²⁵I using Iodogen (Pierce Chemical Co., Rockland, IL)-coated test tubes. Sp act were $\sim 3 \times 10^7$ cpm/ μ g protein.

Antibody-sensitized sheep erythrocytes (SEA) bearing 300 hemolytic sites of guinea pig C1, human C4, and ¹²⁵I-C2 (SEAC14^{oxy2}) were prepared as described (4). Radiolabeled C3b was deposited on SE by incubating labeled C3 with SEAC14^{oxy2} in DGVB²⁺ for 15 min at 30°C. HE were sensitized with a subagglutinating dose of rabbit IgM anti-HE. Antibody-sensitized HE (HEA) were incubated with guinea pig C1 and human C4 in DGVB²⁺ sequentially to form 300 hemolytic sites of C1 and various amounts of C4b per cell (5).

Crosslinking. HE hemolytic intermediates were suspended at 10⁸ cells/ml in PBS (pH 8.3) containing 0.02% sodium azide. The crosslinker dithio-bis(succinimidyl propionate) (DSP) (Pierce Chemical Co.) was dissolved at 10 mg/ml in DMSO and added to cell suspensions at a final concentration of 100 μ g/ml (21). The same amount of DMSO was added to another sample of the cell suspension as a control. Reaction mixtures were incubated, with rotation, for 1 h at room temperature. We stopped the reaction by adding 50 mM Tris-HCl (pH 8.0)-buffered saline and washing the cells with the same buffer. Red cell ghosts were prepared by hypotonic lysis in 15 mM Tris-HCl buffer (pH 7.8), followed by centrifugation at 12,000 *g* for 15 min. The ghosts were dissolved at a concentration corresponding to 5×10^7 cells per 50 μ l in boiling SDS-PAGE sample buffer (5% SDS, 125 mM Tris-HCl buffer [pH 6.8], 10% glycerol, and 0.01% bromphenol blue). Alternatively, the ghosts were extracted with PBS containing 0.5% NP-40 and 1 mM PMSF for 20 min on ice at 10⁸ cells per 100 μ l, followed by centrifugation to remove insoluble materials.

Removal of C4 by Immunoprecipitation. Samples of the NP-40 extract from ghosts of DSP-treated HEAC14 bearing 1,000 molecules of C4b per cell were mixed with 10 μ g of anti-C4 IgG or normal IgG. After 15 min incubation on ice, the mixtures were added to protein A-Sepharose CL-4 B beads (10 μ l) (Pharmacia Fine Chemicals, Piscataway, NJ), followed by 15 min incubation at 20°C. Supernatants were collected after centrifugation, mixed with the SDS-PAGE sample buffer, and analyzed by SDS-PAGE and Western blotting.

Detection of CR1 by Western Blotting. The dissolved ghosts from DSP-treated HE hemolytic intermediates or samples after immunoprecipitation of C4 were subjected to SDS-PAGE on a 4.5% gel. The samples were transferred electrophoretically to nitrocellulose paper using a Bio-Rad transblot system (Bio-Rad Laboratories, Richmond, CA), and excess binding sites on the paper were blocked with BSA. CR1 was detected by incubation with a mixture of anti-CR1 mAbs (57F, 57H, and 44D; 10 μ g/ml each), and then with ¹²⁵I-labeled goat anti-mouse IgG.

Cleavage of HE-bound C4b by Factor I. HEAC14 cells (100 μ l of 10⁸ cells/ml in DGVB²⁺) bearing 1,000 molecules of radiolabeled C4b per cell were mixed with 50 μ l of factor I

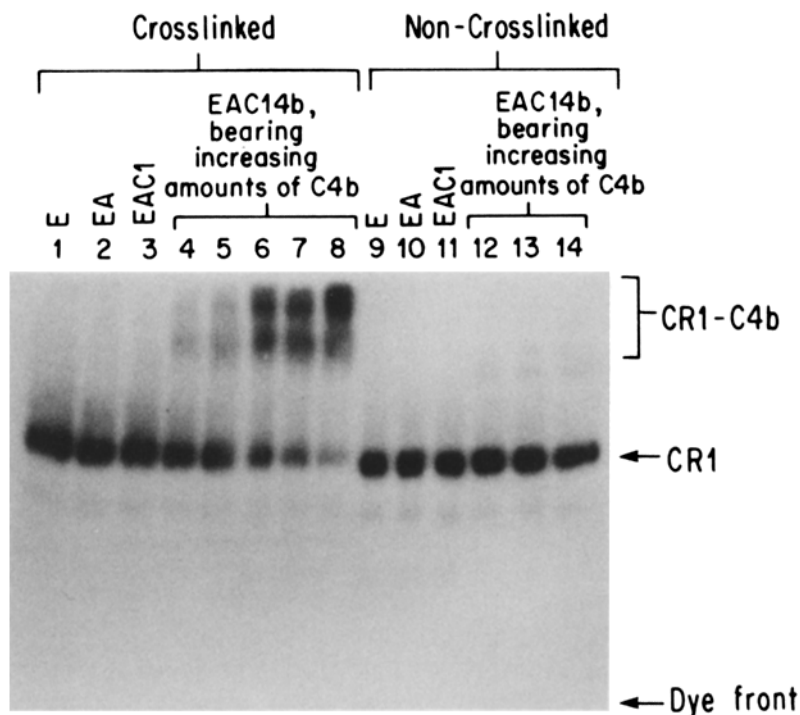


FIGURE 1. Crosslinking of CR1 to C4b on HE. HEAC14 cells, and HE, HEA and HEAC1 as controls were crosslinked with DSP (lanes 1-8) or mock crosslinked (lanes 9-14) and then subjected to SDS-PAGE on a 4.5% gel, and to Western blotting. CR1 was detected on the blot by a mixture of anti-CR1 mAbs followed by labeled second antibodies. Lanes 1 and 9, HE; lanes 2 and 10, HEA; lanes 3 and 11, HEAC1; lanes 4-8, crosslinked HEAC14 bearing 450, 700, 1,000, 1,500, and 1,900 molecules of C4b per cell; lanes 12-14, mock crosslinked HEAC14 bearing 1,000, 1,500, and 1,900 molecules of C4b per cell. High- M_r complexes containing CR1 were seen only in crosslinked HEAC14 (lanes 4-8).

(0-40 $\mu\text{g/ml}$ in DGVB²⁺) and 50 μl of either DGVB²⁺, CR1 (1 $\mu\text{g/ml}$), or HE (6×10^8 cells/ml). After 2 h of incubation at 37°C, 1 ml of DGVB²⁺ was added, and the mixtures were centrifuged. Radiolabeled C4 fragments released into the supernatant were detected in a gamma counter. In another experiment, a mixture of anti-CR1 mAbs was added to HEAC14 and factor I to determine whether the cleavage of C4b was CR1-dependent.

C3c-release Assay for Exogenous CR1 Activity. SEAC1423 cells bearing 1,800 molecules of radiolabeled C3b per cell (5×10^7 cells/ml in DGVB²⁺) were mixed with factor I (5 $\mu\text{g/ml}$) and E^{hu}AC1 bearing various amounts of C4b (5×10^7 cells/ml). After 60 min incubation at 37°C the cells were pelleted by centrifugation and the amount of C3c released into the supernatant was measured by gamma counting.

Results

Formation of CR1-C4b Complexes Within the Cell Membrane. To show the association of CR1 with C4b within the same membrane, a crosslinking reagent (DSP) was used. HEAC14 and control cells, HE, HEA, and HEAC1, were treated with DSP. Western blotting analysis of solubilized cell membranes using anti-CR1 mAbs revealed the presence of free CR1 and of CR1-containing complexes in two wide bands with high M_r (>400,000) (Fig. 1). The high- M_r bands were present only on DSP-treated HEAC14, and not on mock-treated HEAC14 (Fig.

1, lanes 12–14), or on DSP-treated HE, HEA, and HEAC1 (Fig. 1, lanes 1–3). The intensity of the high- M_r bands increased in a dose-dependent fashion with the amount of bound C4b (lanes 4–8). The efficiency of the complex formation was high, that is, most of the CR1 in the red cells was associated with the high- M_r bands when 1,900 molecules of C4b were deposited on HE (Fig. 1, lane 8).

The above results suggested that the high- M_r bands consisted of a complex between CR1 and C4b. To demonstrate this point directly we immunoprecipitated the extracts with anti-C4 antibody and Sepharose–protein A before the Western blotting analysis. As shown in Fig. 2 (lane 1), the high- M_r bands were removed by treatment with anti-C4 but not with normal IgG (Fig. 2, lane 2). As an additional control to show that the complexes between CR1 and C4b were formed within cell membranes rather than between cells, mixtures of HE and SE bearing C4b (SEAC14) were treated with DSP, and the membranes were subjected to Western blotting. Only free CR1 was found in the radioautographs (not shown).

Intrinsic Cofactor Activity of CR1. When HEAC14 cells bearing radiolabeled C4b were incubated at 37°C in the presence of increasing concentrations of factor I, C4b was cleaved and C4 fragments were released into the supernatant in a dose-dependent fashion. The cleavage was CR1-dependent, since mAbs against CR1 (but not against DAF or a malaria parasite antigen) inhibited the activity of I (Fig. 3). The pattern of fragmentation of C4b on HE was identical to that previously observed when SE bearing C4b were treated with factor I and CR1; that is, C4c (M_r 140,000) was released and C4d (M_r 40,000) remained bound to the cell membrane (data not shown).

Additional experiments strongly suggested that the cleavage by I of C4b bound to the human red cells was mediated by the cofactor activity of CR1 within the same cell membrane, rather than that of CR1 on the surface of neighboring cells. For example, the introduction of threefold more HE or 25 times more purified CR1 into reaction mixtures containing HEAC14 and variable concentrations of I did not enhance the extent of cleavage of C4b (Fig. 4a). Neither were the kinetics of release of radiolabeled C4c at a fixed concentration of I (5 μ g/ml) enhanced by purified CR1 or by additional HE (Fig. 4b). Even more striking was the fact that a 10-fold variation in the concentration of HEAC14 had no effect on the extent of cleavage; that is, the reaction was not enhanced by greatly increasing the frequency of cell collisions and encounters between CR1 and exogenous C4b (Fig. 5).

Inhibitory Effect of C4b Deposition on Extrinsic Cofactor Activity of CR1. Next we studied the effect of C4b deposition on the extrinsic cofactor activity of CR1 of human erythrocytes. The assay consisted of measuring the cofactor activity of several different preparations of human red cells; HE, HEC1, HEC14, on the release of C3 fragments (C3c) from SE bearing radiolabeled C3b. As shown in Fig. 6, HE plus I (but not HE, HEC14, or I alone) released >40% of the labeled C3b bound to SE after 1 h of incubation of the mixture at 37°C. HEA or HEAC1 plus I had the same effect as HE plus I. However, after C4 deposition on the human red cells, the cleavage of the exogenous C3b was profoundly inhibited. The inhibitory effect increased with the amount of deposited C4b; for example, 1,800 molecules of deposited C4b inhibited ~50% of the cleavage of

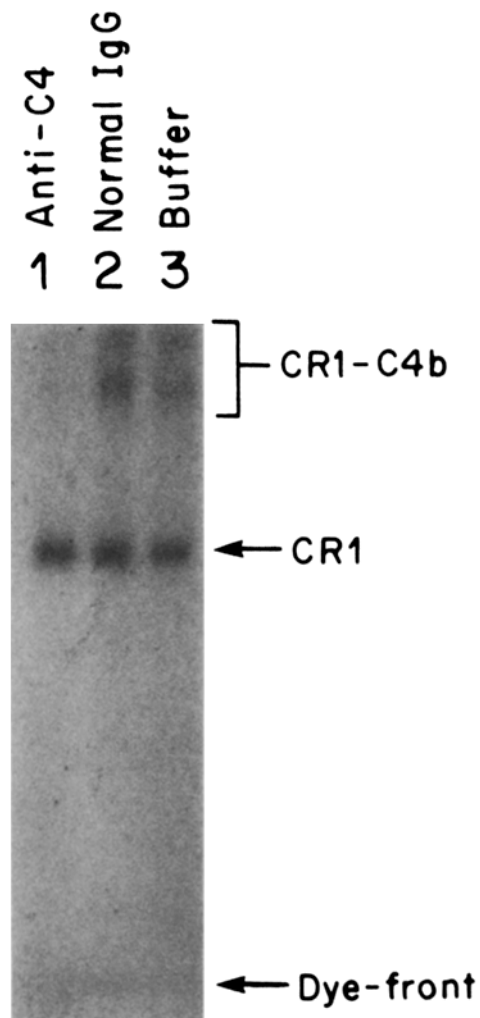


FIGURE 2. Removal of CR1-C4b complexes by immunoprecipitation with anti-C4. The NP-40 extract of DSP-treated HEAC14 was incubated with anti-C4 IgG (lane 1), normal IgG (lane 2) or buffer alone (lane 3). The mixtures were then incubated with protein A-Sepharose beads to remove immune complexes. The beads were separated by centrifugation and the supernatants were subjected to SDS-PAGE and Western blotting as described in Fig. 1. The treatment with anti-C4 IgG removed the high- M_r bands containing CR1, indicating that they originated from the crosslinking of C4b and CR1.

extrinsic C3b, and 3,000 molecules of deposited C4b inhibited the cleavage almost completely.

Discussion

Classical pathway activation of the complement cascade on or close to the surface of red cells leads to the deposition of C4b on their membranes. That this also occurs under physiological conditions is shown by the presence of C4b fragments (C4d) on all normal human or mouse erythrocytes, where they have

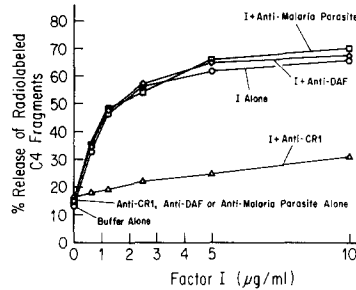


FIGURE 3. CR1-dependent cleavage of HE-bound C4b by factor I. HEAC14 cells bearing 1,000 molecules of radiolabeled C4b per cell (5×10^7 cells/ml in DGVB²⁺) were incubated with increasing amounts of factor I alone (0–10 $\mu\text{g/ml}$) (○), or factor I plus monoclonal anti-CR1 (57F, 57H, and 44D; 12.5 $\mu\text{g/ml}$ each) (△), anti-DAF (IA10, I1H6, and VIIIA7; 12.5 $\mu\text{g/ml}$ each) (◇), or anti-malaria parasite (3D11; 37.5 $\mu\text{g/ml}$) (□). After 2 h incubation at 37°C, the cells were removed by centrifugation and the counts released into the supernatants were measured. Anti-CR1 mAbs inhibited the factor I-mediated release of C4 fragments.

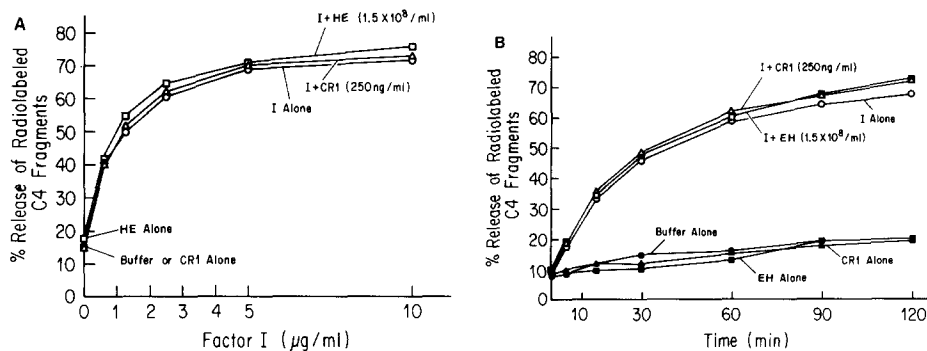


FIGURE 4. Cleavage by factor I of HE-bound C4b is dependent on intrinsic association of C4b with CR1 within the same cell. **A**, HEAC14 cells bearing 1,000 molecules of radiolabeled C4b per cell (5×10^7 cells/ml in DGVB²⁺, containing ~ 10 ng/ml CR1) were incubated with increasing amounts of factor I alone (0–10 $\mu\text{g/ml}$) (○), factor I plus purified CR1 (250 ng/ml) (△), or factor I plus HE (1.5×10^8 cells/ml) (□). After 2 h incubation at 37°C, extent of cleavage of C4b was determined by measuring the release of C4c radioactive fragments. Addition of purified CR1 or HE did not enhance the cleavage. **B**, HEAC14 cells bearing 1,000 molecules labeled C4b per cell (5×10^7 cell/ml) were incubated at 37°C with factor I alone (5 $\mu\text{g/ml}$) (○), factor I plus purified CR1 (250 ng/ml) (△), factor I plus HE (1.5×10^8 cells/ml) (□), DGVB²⁺ alone (●), CR1 alone (▲), or HE alone (■). At different time intervals, the extent of cleavage of C4b was determined. Addition of purified CR1 or HE had no significant effect on the rate of cleavage.

been identified as blood group antigens (22, 23). The mechanisms that regulate the function of membrane-bound C4b and which lead to its fragmentation on autologous cell membranes are poorly understood. Although DAF prevents the binding of C2 to C4b and the formation of the C3-convertase C4b, 2a, it has no effect on the structure of C4b (4).

A few studies have dealt with the fate of C4b bound to heterologous cells. The α' chain of human C4b deposited on the membrane of SE can be cleaved by the serum enzyme I (20). However, during the initial phases of activation of the cascade when the number of deposited C4b molecules is small, the fragmentation of C4b by I is inefficient (24). The activity of I can be enhanced by the serum

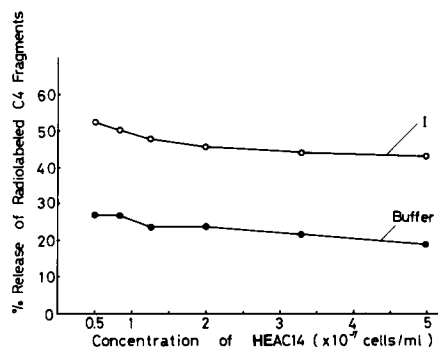


FIGURE 5. Effect of varying the concentration of HEAC14 cells on the C4b cleavage when the concentration of factor I is kept constant. 10^7 cells of HEAC14 bearing 1,000 molecules of radiolabeled C4b were suspended in various volumes (0.2–2 ml) of DGVB²⁺ containing factor I at a concentration of 1.5 $\mu\text{g}/\text{ml}$ (○) or DGVB²⁺ (●). After 1 h incubation at 37°C, the amounts of radiolabeled C4b fragments released were determined. The cleavage of C4b was not enhanced at higher concentration of the cells, suggesting that the cleavage was not influenced by the frequency of cell collisions.

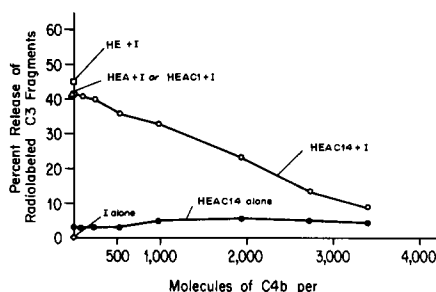


FIGURE 6. Inhibition by intrinsic C4b of the extrinsic cofactor activity of CR1. SEAC1423 cells (5×10^7 cells/ml in DGVB²⁺) bearing 1,800 molecules of radiolabeled C3b per cell were incubated for 1 h at 37°C with HEAC14 cells (5×10^7 cells/ml) bearing increasing amounts of C4b in the presence (○) or absence (●) of factor I (5 $\mu\text{g}/\text{ml}$). The number of bound C4b molecules in each cell intermediate was calculated from the results of preliminary experiments in which the same concentrations of radiolabeled C4 were used. HE, HEA, and HEAC1, either alone or with factor I, were included as controls. In the presence of factor I plus HE, HEA, or HEAC1, ~42% of C3 radiolabel was released. The cofactor activity of HEAC14 cells decreased and the decrease was dependent on the dose of C4b bound per cell.

cofactor C4bp, or by the extrinsic activity of human CR1, but this only occurs when the number of bound C4b on SE is high, or after amplification of the cascade and C3b deposition (24, 25).

The main new findings of this paper are that small numbers of human C4b molecules deposited on human erythrocytes bind to endogenous CR1, and that the bound C4b is cleaved by serum factor I. The intrinsic association between CR1 and the membrane-bound C4b was demonstrated by the formation of complexes between the two molecules when HEAC14 were treated with a bifunctional crosslinking reagent (DSP).

The substrate for the enzymatic activity of I was primarily the C4b bound endogenously to CR1, and not C4b bound to CR1 present on the surface of a different HEAC14 cell. Indeed, the kinetics or the extent of cleavage of C4b were

not affected by greatly increasing the concentration of extrinsic CR1 in the incubation mixture by adding either HE or purified CR1. In these experiments, we purposely deposited limited amounts of C4b on the red cells (a maximum of 1,000 molecules), in order to better mimic the initial phases of complement activation *in vivo*. It is likely that extrinsic CR1 would also act on HEAC14 if a larger number of C4b molecules had been deposited on the human red cells. Furthermore, the extent of cleavage was not affected by increasing the concentration of HEC14 cells and therefore the frequency of cell collisions.

Most of the CR1 of the erythrocytes could be crosslinked to C4b when 1,900 molecules of C4b were deposited on the red cell surface. Although a relatively short time (30 min at room temperature) elapsed between the deposition of C4b and the addition of DSP, the efficiency of crosslinking was high. This suggests that the encounters (presumably random) between CR1 and C4b led to the formation of stable CR1-C4b complexes. The high avidity of deposited C4b for endogenous CR1 is also highlighted by the concomitant loss of the extrinsic cofactor activity of the complement receptor for exogenous target-bound C3b (Fig. 6). This is unexpected, since an HE contains only several hundred molecules of CR1, which have much higher binding avidity for C3b than for fluid-phase C4b (20) or C4b deposited extrinsically onto SE (26). Nonetheless, it is conceivable that other factors enhance the binding of C4b to CR1 on the surface of the lipid bilayer, *i.e.*, the geometry of the respective binding domains may favor the interaction, or alternatively, the dissociation of the formed complexes may be restricted by secondary events or by other neighboring molecules.

The precise composition of the CR1-C4b complexes is unknown. When extracts of membranes of the DSP-treated HEAC14 cells were subjected to SDS-PAGE, two high- M_r bands, containing C4b and CR1, were detected by Western blotting. One band had $M_r \sim 400,000$, and must contain only one molecule each of CR1 ($\sim 200,000 M_r$) and C4b ($190,000 M_r$). The second band, of higher M_r , could represent complexes between C4b clusters with CR1, or could contain additional membrane molecules. Although DAF can also bind intrinsically to C4b, it is unlikely that it is included in this band. Using the same methods, we found that most of the DAF-C4b complexes have M_r of 250,000–300,000 (11). Moreover, the DAF-C4b complexes formed on human red cells were only detected by this technique when the number of deposited C4b molecules was $>3 \times 10^3$ per cell (11), while the C4b-CR1-containing bands could be seen in cells bearing 10 times less C4b.

On the basis of the present and prior observations, we speculate (Fig. 7) that both DAF and CR1 act endogenously to inactivate the function of C4b and prevent the progression of the cascade. DAF, which is anchored on the cell membrane via phosphatidylinositol (27, 28), may move more rapidly than CR1 on the plane of the membrane. DAF binding prevents the formation of the C3 convertase C4b_{2a}. The cleavage of C4b and the generation of the inactive C4d fragments (that is, the Chido and Rodgers blood groups [22]) occurs after the concerted activities of endogenous CR1 and serum factor I. Because neither DAF nor CR1 has detectable binding affinity for the remaining membrane-bound fragment, C4d, we presume that the membrane inhibitors will become free to interact with additional ligands. This hypothesis does not distinguish

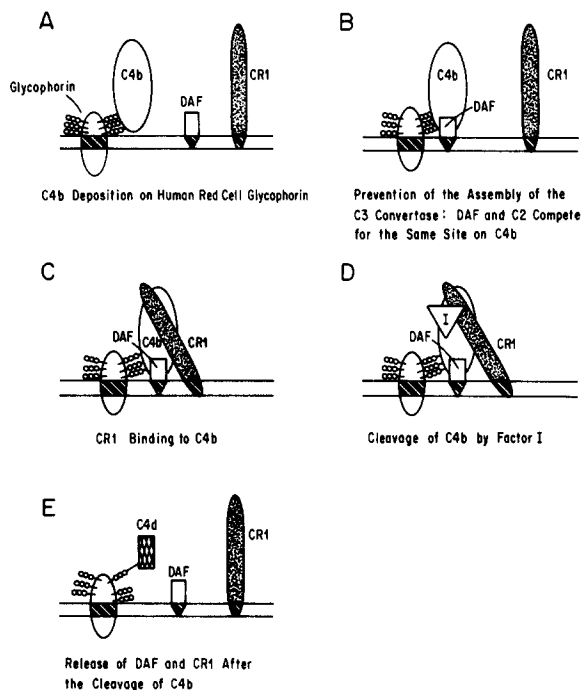


FIGURE 7. Schematic representation of the proposed mechanism of processing of autologous erythrocyte-bound C4b by complement regulatory proteins. The CR1 bound to C4b is shown slightly tilted only to simplify the drawing of the complexes.

between the fate of the two isotypes of C4b (29) nor take into account the fact that they may form covalent bonds with different structures of the acceptor membrane molecules (30–32).

Others have shown that C3b bound to human erythrocytes can be attacked by I, and that this reaction is CR1 dependent, but their experiments did not distinguish between the intrinsic and extrinsic activities of the receptor (1). Because of the central role of C3b in initiation and amplification of the alternative pathway, and the observed deficiencies of CR1 in some autoimmune diseases (33–35), this question has considerable interest. In the light of the present findings and the fact that CR1 has higher binding affinity for extrinsically bound C3b than for C4b, it seems likely that CR1 will also function endogenously to inactivate C3b. This point, however, remains to be established.

Summary

Activation of the classical complement pathway on the membrane of autologous cells results in the deposition of C4b on their surface and in the assembly of the C3 convertase C4b2a, one of the amplifying enzymes of the cascade. Here we study the sequence of events leading to irreversible inactivation of the potentially harmful C4b bound to human red cells. We show that deposited C4b interacts endogenously with complement receptor type 1 (CR1) present on the membrane of the same red cell. Complexes containing CR1 and C4b are found

in extracts of membranes of C4b-bearing red cells after treatment of the intact cells with a bifunctional crosslinking reagent. The amount of complexed CR1 increases with the number of deposited C4b molecules. Only small amounts of free CR1 are observed on red cells bearing as few as 1,900 molecules of C4b, suggesting that the binding avidity between C4b and endogenous CR1 is high. In agreement with this observation, we find that the deposited C4b inhibits the exogenous cofactor activity of the red cell CR1 for the factor I-mediated cleavage of target-bound clustered C3b.

The C4b bound to the human red cells is cleaved by the serum enzyme C3b/C4b inactivator (factor I) and a large fragment (C4c) is released in the incubation medium. The cleavage is totally inhibited by mAbs against CR1, showing that the complement receptor is an essential cofactor for the activity of I. When the number of bound C4b per red cell is relatively small (<1,000 molecules) the substrate for the enzymatic activity of factor I is mostly or exclusively the C4b bound endogenously to CR1. Indeed, the kinetics or the extent of cleavage of C4b are not affected by greatly augmenting the concentration of exogenous CR1 or of C4b-bearing red cells in the incubation mixture, thereby increasing the frequency of collisions between CR1 on the surface of one cell with C4b deposited on the membrane of a different cell.

On the basis of the present and prior observations, we speculate that both DAF and CR1 act endogenously to inactivate the function of autologous red cell-bound C4b and prevent the progression of the cascade. DAF binding prevents the formation of the C3 convertase, C4b2a. The cleavage and irreversible inactivation of C4b only occurs after the concerted activities of endogenous CR1 and serum factor I. Because the remaining membrane-bound C4d fragments (that is, the Chido and Rodgers blood groups) have no demonstrable binding affinity for DAF or CR1, these membrane proteins will be free to interact with additional bound ligands.

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