CONTROL OF THE FUNCTION OF SUBSTRATE-BOUND C4b-C3b BY THE COMPLEMENT RECEPTOR CR1

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It has been recently reported (1–3) that the complement receptor for C3b (CR1) has a unique role in the degradation of C3b bound to the cell membrane or to immune complexes (*C3b). CR1 can be more effective (4) than serum factor H in promoting the I-mediated transformation of *C3b into the hemolytically inactive intermediate *iC3b (5). Moreover, under physiological conditions, CR1, but not H, serves as a cofactor for the further cleavage of *iC3b into C3c and *C3dg, the ligand for the C3d receptor (CR2) (1–3). These findings, in conjunction with other evidence (6–10), have been interpreted as meaning that the processing of *C3b-containing antigen-antibody complexes in vivo occurs on the surface of red cells (which bear most of the CR1 in human peripheral blood), and as indicative that the CR1 deficiencies observed in patients with systemic lupus erythematosus and rheumatoid arthritis (11–15) might be involved in the pathogenesis of these immune complex diseases.

When the complement cascade is triggered by the classic pathway, both *C3b and *C4b bind covalently to the activating substrate and participate in formation of the enzymes C3-convertase (C4b,2a) and C5-convertase (C4b,2a,3b), which in turn augment the cascade reaction (reviewed in 16). Since *C4b, which regulates the activity of both enzymes, is also a ligand for CR1 (17, 18), and CR1 can inhibit both enzymes (19), we study here the effects of the interaction between red cell intermediates bearing C3 and C5 convertases and CR1 in the presence of serum factor I. We present evidence not only that CR1 may be a principal cofactor responsible for the physiological degradation of *C4b, but also that the inactivation of *C4b can be greatly enhanced when sufficient *C3b is deposited in its vicinity. Likewise, the inactivation of *C3b is enhanced by adjacent *C4b.

Materials and Methods

Buffers, Reagents, and Proteins. Isotonic veronal-buffered saline ($DGVB^{++}$)[1] used in most studies contained 2.5 mM veronal, pH 7.4, 73 mM NaCl, 2.5% dextrose, 0.15 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1% gelatin. Isotonic veronal buffer (GVB^{++}) used in some

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[1] *Abbreviations used in this paper:* $DGVB^{++}$, isotonic veronal-buffered saline containing 2.5 mM veronal, pH 7.3, 73 mM NaCl, 2.5% dextrose, 0.15 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1% gelatin; E^{hu}, human erythrocytes; GVB^{++}, isotonic veronal buffer prepared as $DGVB^{++}$ except with 146 mM NaCl instead of 73 mM NaCl and dextrose; KSCN, potassium thiocyanate; NP-40, Nonidet P-40; PBS, phosphate-buffered saline; SFU, site-forming unit; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

experiments contained 146 mM NaCl instead of 73 mM NaCl and 2.5% dextrose. Metal-chelating veronal buffer (GVB-EDTA), used in hemolytic assays and for washing cells, contained 10 mM EDTA and lacked $CaCl_2$ and $MgCl_2$.

C4 and C3 were purified from DEAE-Sephacel fractions of pooled human plasma by polyethylene glycol precipitation followed by DEAE-Sephacel rechromatography or Sephadex G-200 gel filtration, respectively (20). C4 contained no C3 or C5 hemolytic activity, and its specific activity was 1.1×10^6 C4 site-forming units (SFU) per mg protein immediately after purification. It was stored at 4°C in sterile tubes containing 25 mM benzamidine. C3 was depleted of residual C5 and H activities by adsorption with Sepharose anti-human C5 and anti-human H, respectively. The specific activity of C3 was 8×10^3 SFU/mg. It was stored frozen in aliquots at −70°C. C2 (21) and C5 (20) were purified as described, and in some experiments C2 was used in an oxidized form (^{oxy}C2) (22). C1 (23), C6-9 (24), and C-EDTA were prepared from guinea pig serum. C4b was prepared by "spontaneous" conversion of C4 upon removal of benzamidine by dialysis.

I (25), C4bp (26), H (20), and CR1 (5, 19) were purified as described. I was also obtained commercially (Cordis Laboratories Inc., Miami, FL). C4bp was depleted of contaminating IgM and H by adsorption with Sepharose anti-IgM and anti-H, respectively. The final product consisted predominantly of the higher molecular weight form (26). H was depleted of contaminating IgA by adsorption with Sepharose-anti-IgA. For the purification of CR1, 0.1% Nonidet P-40 (NP-40) extracts of human erythrocyte (E^{hu}) stroma were chromatographed on Biorex-70 (Bio-Rad Laboratories, Richmond, CA) as described (5). After salt gradient elution, the fractions containing CR1 (as determined by their inhibitory activity on C5-convertase, C4b,2a,3b) were pooled, concentrated, and filtered through Sepharose-anti-CR1 prepared with two monoclonal antibodies (44D and 57H) (12, 19). After washing, the bound CR1 was eluted with 3 M potassium thiocyanate (KSCN). Excess KSCN was removed by passage of the eluate through Sephadex G-25 equilibrated in phosphate-buffered saline (PBS) containing 0.1% NP-40. The final product contained negligible impurities as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions followed by silver staining (Bio-Rad Laboratories).

E^{hu} were purified from acid/citrate/dextrose-treated venous blood samples from healthy volunteers. Cells were washed five times with large volumes of GVB-EDTA and buffy coats aspirated to remove white blood cells. The remaining E^{hu} were washed twice in $DGVB^{++}$ and resuspended to appropriate concentration.

SDS-PAGE. SDS-PAGE was performed as described by Laemmli (27). Stacking gels of 3% and running gels of 7.5% were used. Samples were reduced by the addition of 20% 2-mercaptoethanol to the sample buffer. Molecular weights were estimated by comparison with a set of commercially available standards (Bethesda Research Laboratories, Rockville, MD). Gels were fixed and stained with methanol/acetic acid containing Coomassie Blue. Radioautographs were exposed at −70°C using X-Omat R film (XR-5; Eastman Kodak Co., Rochester, NY).

Radiolabeling. C4 and C3 were labeled with ^{125}I or ^{131}I using Iodogen (Pierce Chemical Co., Rockford, IL) as per the manufacturer's instructions. Unbound ^{125}I or ^{131}I was eliminated by gel filtration through Sephadex G-25 QS-2A Quick-Sep columns (Isolab, Inc., Akron, OH) followed by dialysis.

Cellular Intermediates and Hemolytic Assays. Sheep erythrocytes (E) were sensitized with rabbit anti-sheep hemolysin (A) (Gibco Laboratories, Grand Island, NY) and the resulting EA washed and adjusted to 1×10^8 cells/ml in $DGVB^{++}$. An equal volume of C1 at a dilution sufficient to provide 300 SFU was added and the mixture incubated 15 min at 30°C. After washing, the intermediates (EAC1) were incubated for 20 min at 30°C with an equal volume of an appropriate dilution of C4 to yield cells (EAC14) bearing the amounts of *C4b specified in the text or figure legends. EAC14, 1×10^8/ml in $DGVB^{++}$, were incubated for 5 min at 30°C with 300 SFU/ml of C2 (or ^{oxy}C2) and the intermediates (EAC142 or EAC14^{oxy}C2) washed, readjusted to 1×10^8/ml in $DGVB^{++}$, and incubated with an equal volume of C3 at a dilution sufficient to yield cells (EAC1423 or EAC14^{oxy}23) bearing the specified amount of *C3b. C4b hemolytic sites were determined by sequential

incubation of EAC14 with 300 SFU of ^{oxy}C2 for 5 min at 30°C and C-EDTA for 1 h at 37°C. C3b hemolytic sites were determined by incubation of EAC1423 (or EAC14^{oxy}23) with 300 SFU of C5 for 5 min at 30°C and C6-9 for 1 h at 37°C. The number of *C4b or *C3b molecules deposited on the erythrocytes was calculated from the percentage of uptake of samples containing ^{125}I-C4 or ^{125}I-C3.

C4c and C3c Release. EAC14 bearing ^{125}I-labeled *C4b were added in 25-μl aliquots to reaction tubes containing 25-μl volumes of I (or DGVB^{++} as control) and 50 μl volumes of different dilutions of C4bp, CR1, or E^{hu} (or DGVB^{++} as control). The final concentrations of the reactants are given in the text or figure legends. The tubes were incubated at either 37 or 30°C in a water bath and agitated periodically. The reaction was stopped by transfer of the tubes to an ice bath and addition of 900 μl of ice-cold DGVB^{++}. The cells were pelleted and 500 μl of supernatant was removed. The percentage of released C4 label was calculated from the counts present in the separated 500-μl fraction and those remaining in the tube. Studies on the release of C4c or C3c from EAC1423 bearing ^{125}I-labeled *C3b or bearing both ^{125}I-labeled *C4b and ^{131}I-labeled *C3b were performed as described above.

Results

*Ability of CR1 to Promote I-mediated Degradation of Substrate-bound *C4b*

Previous studies (28) have indicated that the enhancing activity of C4bp on the I-mediated degradation of *C4b is observed only when the *C4b density reaches at least 3×10^3 molecules per cell. To determine the effect of *C4b density on CR1 activity, release of C4 label by I plus CR1 was studied using EAC14 prepared with different amounts of C4. As shown in Fig. 1, the cofactor activity of CR1 was not observed at low *C4b densities, but increased progressively when the number of *C4b molecules surpassed 3×10^3 per cell.

The effects of C4bp, of purified CR1, and of intact E^{hu} on the I-mediated breakdown of cell-bound *C4b into C4c and *C4d were then compared. In view of the results shown in Fig. 1, these studies were performed using a cell intermediate EAC14 bearing 6×10^3 ^{125}I-*C4b molecules per cell. Cells (5×10^7/ml in DGVB^{++}) were mixed with I (16 μg/ml) plus CR1, E^{hu}, or C4bp, in increasing concentrations, or with each factor alone as control. After incubation at 37°C for 60 min, the reactions were stopped and the percentage of C4

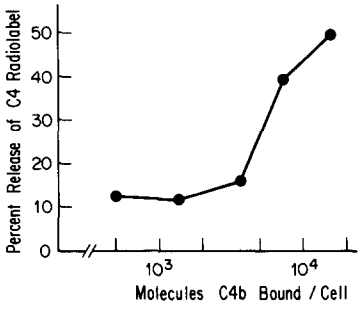

FIGURE 1. Effect of *C4b density on the release of C4 fragments from EAC14. EAC14 (5×10^7/ml) bearing increasing amounts (5×10^2; 1.4, 3.7, or 7.3×10^3 or 1.6×10^4 molecules/cell) of ^{125}I-*C4b were incubated at 37°C for 1 h with 16 μg/ml of I and 800 ng/ml of CR1. The percentage of release of ^{125}I-C4 radiolabel is shown as a function of the density of deposited ^{125}I-*C4b on the cells. About 10% release was observed in the presence of I alone at all *C4b densities. CR1 enhanced the release of C4 fragments only at *C4b densities $>3 \times 10^3$ molecules per cell.

radiolabel released into the supernatant was determined. CR1, E^{hu}, and C4bp all enhanced I-mediated release of radiolabel in a dose-dependent fashion (Fig. 2). As little as 50 ng/ml of purified CR1 or 6.2×10^7 E^{hu}/ml (containing ~60 ng/ml of CR1) caused significant enhancement. In contrast, as much as 30 μg/ml of C4bp had substantially less effect. Minimal release occurred in the presence of CR1, E^{hu}, or C4bp alone.

To verify whether the release observed in the presence of CR1 or E^{hu} was mediated in fact by CR1 rather than by some other factor, additional studies were performed in which a pool of purified monoclonal anti-CR1 antibodies (44D, 31D, 57F) was added to the reaction mixtures. Parallel studies were also performed in which E^{hu} were replaced by erythrocytes from animal species known not to mediate immune adherence of EAC1423. As also shown in Fig. 2, the CR1- or E^{hu}-mediated release of C4 label was strongly inhibited by monoclonal antibodies to CR1. Minimal release was observed in the presence of as many as twofold higher numbers of erythrocytes from guinea pigs or sheep (not shown).

*Modulation by *C3b of I plus CR1-mediated *C4b Degradation.* In the presence of C3, *C4b,2a-bearing substrates can rapidly fix *C3b. Since CR1 binds to *C3b in addition to *C4b, we next studied the effect of deposited *C3b on the degradation of substrate-bound *C4b by I plus CR1. EAC14 (5×10^7/ml) prepared with radiolabeled ^{125}I-C4 and bearing $\sim 3 \times 10^3$ molecules of *C4b per cell were incubated with excess C2. Then increasing amounts (3–81 SFU/ml) of unlabeled C3 (or $DGVB^{++}$ as control) were added. The number of *C3b molecules deposited at each C3 input was calculated from parallel experiments in which EAC14 were prepared with unlabeled C4 and the cells incubated with ^{125}I-C3. The resulting cells bearing 3×10^3 molecules of ^{125}I-labeled *C4b and from 0 to 5×10^4 molecules of *C3b were then incubated at 37°C for 60 min with I (16 μg/ml) and increasing concentrations of CR1 (100–800 ng/ml). In a second set of tubes, cells were incubated with the same concentration of I and increasing concentrations of C4bp in comparable blood proportions (2.5–20 μg/

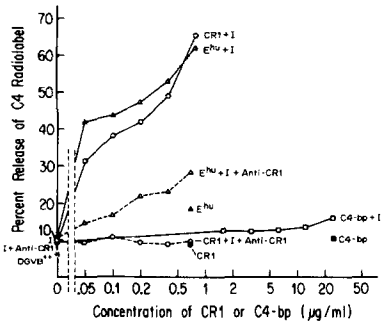

FIGURE 2. Comparative activities of C4bp, CR1, and E^{hu} in promoting the I-mediated release of C4 fragments. EAC14 (5×10^7/ml) bearing 6×10^3 ^{125}I-*C4b molecules per cell were incubated at 37°C for 60 min with I (16 μg/ml) plus C4bp, purified CR1, or E^{hu} membrane-associated CR1 in increasing concentrations, or alternatively with I, $DGVB^{++}$, C4bp, CR1, or E^{hu} alone as controls. Release of C4 radiolabel is shown as a function of cofactor input in μg/ml. About 10^3-fold more C4bp than purified or membrane-associated CR1 was required for comparable enhancement of release and no significant release occurred in the absence of I. Addition of a pool of three monoclonal anti-CR1 antibodies (31D, 44D and 57F), each at 15 μg/ml, strongly inhibited CR1- and E^{hu}-mediated enhancement of release.

ml). The percentages of C4 radiolabel released from the red cells are shown in Fig. 3. Clearly, *C3b fixation enhanced the I plus CR1–mediated C4 release in a dose-dependent fashion. Maximum enhancement of CR1 activity (5–10-fold in different experiments) was obtained with cells bearing 2×10^4 molecules of *C3b at a *C3b/*C4b molar ratio of ~8:1. In contrast, in this and other experiments, *C3b suppressed the I plus C4bp–mediated release of C4 radiolabel. Maximum suppression of approximately two- to fourfold was observed with cells bearing 5×10^4 molecules of *C3b at a *C3b/*C4b molar ratio of 17:1. The release of C4 label by I alone was also inhibited by *C3b.

The *C3b-mediated enhancement of the activity of CR1 on *C4b degradation had two interesting and perhaps physiologically significant consequences. First, this enhancement was also observed when *C4b densities were low. In Fig. 4, we show the effect of I plus CR1 on EAC1423 bearing only 1×10^3 molecules of *C4b but increasing numbers of *C3b per cell. Clearly *C3b promoted C4 release from the EAC1423 cells and the effect was proportional to the amount of *C3b fixed. This result should be contrasted with those of Fig. 1 and of Fujita and Tamura (28) showing that CR1 and C4bp only enhance the activity of I on EAC14 if the density of *C4b is $>3 \times 10^3$ molecules per cell.

Second, we found that after *C3b fixation, CR1 was active on *C4b even in the presence of C2, which, in previous studies (19), has been shown to competitively inhibit the binding of CR1 to *C4b. Our studies were performed as follows. EAC142 bearing 3×10^3 molecules of ^{125}I-*C4b per cell and saturating amounts of C2a were incubated with DGVB^{++} or with C3 at a dilution sufficient to yield

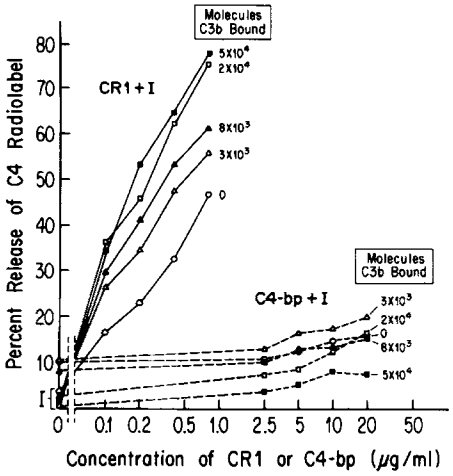

FIGURE 3. Effect of fixed *C3b on the I plus CR1- and I plus C4bp–mediated release of substrate-bound C4 fragment. EAC14 (5×10^7/ml) bearing 3×10^3 molecules of ^{125}I-*C4b per cell were incubated stepwise with 300 SFU of C2 and then either with DGVB^{++} or with increasing amounts of C3. The resulting cellular intermediates bearing a constant amount of ^{125}I-*C4b and the respective numbers of (unlabeled) *C3b molecules indicated in the figure were then incubated at 37°C for 60 min with 16 μg/ml I plus increasing concentrations of CR1 or with the same concentration of I plus comparable proportions of the normal blood content of C4bp. The percentage of release of C4 radiolabel is shown as a function of CR1 or C4bp input. Release by I plus CR1 increased as the amount of deposited *C3b was increased, whereas release by I plus C4bp generally decreased.

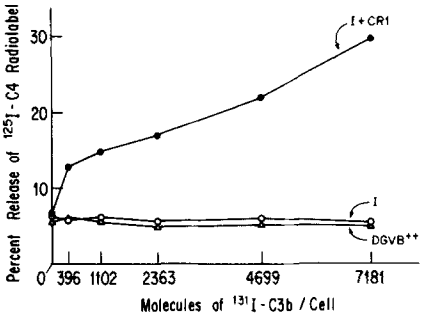

FIGURE 4. Ability of CR1 to augment the I-mediated *C4b fragmentation at low *C4b density when *C3b is present on the same cells. EAC14 (5×10^7/ml) bearing 1×10^3 molecules of ^{125}I-*C4b per cell were incubated with 300 SFU of C2 and then with increasing amounts of ^{131}I-labeled C3. The resulting EAC1423 bearing either 0, 4×10^2, or 1.1, 2.4, 4.7, or 7.2 $\times 10^3$ molecules of *C3b per cell were then incubated at 37°C for 30 min with I (16 μg/ml) and CR1 (800 ng/ml). Release of ^{125}I-C4 radiolabel is shown as a function of density of ^{131}I-*C3b on the cells. The release increased in a dose-dependent fashion with *C3b density. The release in the presence of I (16 μg/ml) alone was not significantly greater than that in the presence of DGVB^{++}

EAC1423 bearing an eightfold molar excess of unlabeled *C3b (2.4×10^4 molecules per cell). After decay of original C2a from the DGVB^{++}-treated EAC142 and from the EAC1423, the cells were reincubated with multiple dilutions of C2. The resulting intermediates, EAC142 and EAC1423 (5×10^7/ml) bearing various amounts of new C2a, were then incubated at 37°C for 60 min with I (16 μg/ml) and CR1 (200 ng/ml) and the percentage of release of C4 radiolabel determined. The results are shown in Fig. 5. Reincubation with large amounts of C2 (2,500 SFU) significantly inhibited the release of C4 label from EAC142. Prior presence of *C3b, however, at a *C3b/*C4b molar ratio of 8:1, reversed the C2-mediated inhibition.

It is also of interest to note that in previous studies (28–30) relatively high concentrations of the control proteins I and C4bp and 1–2 h of incubation at 37°C have been required to release significant amounts of C4c from EAC14. After fixation of optimal amounts of *C3b, however, we observed significant release of C4 label after incubation for only 30 min at 30°C in the presence of as little as 2 μg/ml of I and 25 ng/ml of CR1. As expected, under these conditions there was minimal release of C4 fragments in the presence of I alone or I plus C4bp.

*Evidence that *C4b Can Enhance the I plus CR1-mediated Degradation of *C3b on the Cell Intermediate EAC1423.* To examine the influence of *C4b on the CR1-mediated degradation of neighboring *C3b, we used guinea pig C4 (4 SFU) and C2 (300 SFU) and a concentration of ^{125}I-human C3 predetermined to yield the intermediate EAC14^{gp}2^{gp}3^{hu} bearing limited amounts of C4^{gp} and C3^{hu} (1.5×10^3 molecules/cell). The cells were washed and after decay of C2a and incubation with additional C1, different portions of the EAC14^{gp}3^{hu} cells were reincubated with increasing amounts of human C4. The EAC143, bearing a constant and limited amount of both ^{125}I-*C3b^{hu} and *C4b^{gp}, but increasing amounts of *C4b^{hu}, were then incubated with I plus CR1 as above. As can be seen in Fig. 6, the release of C3c was enhanced in a dose-dependent fashion by the addition of

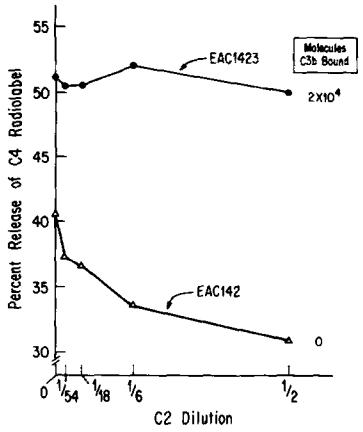

FIGURE 5. Inhibitory effect of C2a on the degradation of *C4b and reversal of the C2a effect by *C3b. EAC142 (5×10^7/ml) bearing 3×10^3 molecules of ^{125}I-*C4b per cell and prepared with 100 SFU of C2 were incubated with either DGVB^{++} or with 30 SFU of unlabeled C3. The resulting cells (5×10^7/ml) bearing the same number of ^{125}I *C4b molecules and either 0 or 2.4×10^4 molecules of *C3b were then decayed at 30°C for 60 min to remove residual C2a, reincubated for 5 min at 30°C with multiple dilutions of fresh C2, and, immediately after washing, incubated for 60 min at 37°C with I (16 μg/ml) and CR1 (200 ng/ml). The percentage of release of C4 radiolabel is shown as a function of the amount of new C2 added in the absence or presence of *C3b on the cells. In the absence of *C3b, C2a inhibited I plus CR1–mediated release of C4 radiolabel in a dose-dependent fashion. The presence of 2.4×10^4 molecules of *C3b not only enhanced release but also reversed the C2a effect.

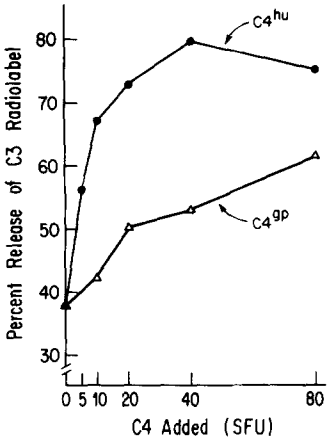

FIGURE 6. Enhancing effect of *C4b on *C3b fragmentation by I plus CR1 at low *C3b density and comparative activities of human and guinea pig *C4b. EAC1423 were prepared with 5 SFU of C4^{gp}, 300 SFU of C2^{gp}, and sufficient human ^{125}I-C3 (20 SFU) to deposit 1.5×10^3 molecules per cell of ^{125}I *C3b. The EAC14^{gp}2^{gp}3^{hu} were then decayed by incubation in DGVB^{++} for 60 min at 30°C to remove C2a and reincubated with additional C1 (300 SFU) and then increasing amounts of C4^{hu} or C4^{gp}. The resulting cells bearing a small amount of *C4b^{gp} and a constant amount of ^{125}I-*C3b but increasing amounts of *C4b^{hu} or additional *C4b^{gp}were then incubated at 30°C for 30 min with I (2 μg/ml) and CR1 (300 ng/ml). The percentage of release of C3 radiolabel is shown as a function of the amount of C4^{hu} or additional C4^{gp} added to the cells. The release increased with C4 input and release from cells bearing human *C4b was greater than that from cells bearing guinea pig *C4b.

*$C4b^{hu}$. A similar experiment was performed in which the same $EAC14^{gp}3^{hu}$ were reincubated with increasing amounts of $C4^{gp}$. In this case the enhancement of release was less marked, most likely because *$C4b^{gp}$ reacts less efficiently with human CR1.

To obtain further evidence that *C4b enhances the degradation of *C3b, we studied the effects of C2 (which interacts with *C4b) and of antibodies to C4. EAC1423 bearing unlabeled *C4b and labeled *C3b were prepared in a conventional fashion using 300 SFU $C4^{hu}$ and 5 SFU ^{125}I-labeled $C3^{hu}$. The cells were incubated at 30°C for 60 min to decay residual C2a. Then the resulting EAC143 were incubated with increasing amounts of C2 (100–2,500 SFU), or alternatively, with increasing amounts of IgG from a goat antiserum to human C4. After washing, the cells (5×10^7/ml) were incubated at 30°C for 30 min with I (2 μg/ml) and CR1 (100 ng/ml) and the percentage of release of C3 radiolabel measured. As shown in Fig. 7, treatment of EAC143 with C2 or exposure to anti-C4 antibody inhibited C3c release. Assuming that 10% of the immunoglobulin in the antiserum is anti-C4, we calculate that 1–2 antibody molecules per *C4b molecule were sufficient to inhibit *C3b fragmentation.

The effect of *C4b on I plus CR1–mediated fragmentation of EAC1423i was next examined. The rationale for these experiments was that *iC3b has low affinity for CR1, and therefore *iC3b degradation might also be greatly enhanced by neighboring *C4b. EAC1423 were prepared bearing limited *C4b (4 SFU of C4) and ^{125}I-*C3b (3.5×10^3 molecules/cell) and incubated at 30°C for

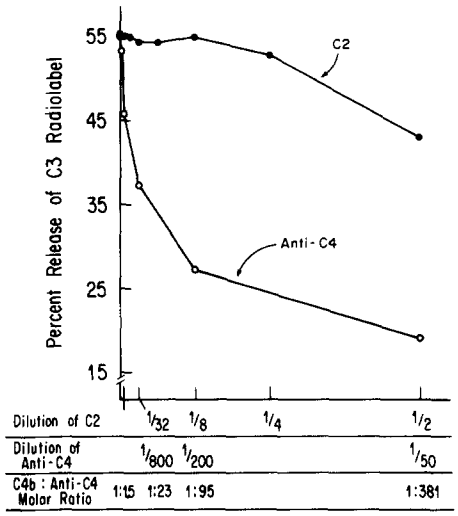

FIGURE 7. Inhibitory effect of anti-C4b antibodies and of C2 on *C3b fragmentation. EAC1423 were prepared with 300 SFU of unlabeled C4 and limited (~5 SFU) of ^{125}I-C3. The resulting cells bearing ~3×10^5 molecules of unlabeled *C4b and ~3×10^5 molecules of ^{125}I-*C3b were decayed at 30°C for 60 min to eliminate residual C2a and then incubated for 5 min at 30°C with decreasing dilutions of human C2 or goat anti-C4 globulin. Immediately after washing, the cells were further incubated at 30°C for 30 min with I (2 μg/ml) and CR1 (100 ng/ml). The percentage of release of C3 radiolabel is shown as a function of the C2 or anti-C4 dilution used to pretreat the cells. Under these conditions of limited *C3b, both C2a and anti-C4 inhibited the release of C3 radiolabel. The inhibitory effect of anti-C4 was more profound than that of C2a.

30 min with I (2 μg/ml) and H (10 μg/ml) to obtain EAC1423i. Hemolytic assays revealed >90% abrogation of the initial C3b hemolytic activity on these cells. The cells were then reincubated with excess C1 (300 SFU) and increasing amounts of C4. The cells were subsequently mixed with I (2 μg/ml) and increasing concentrations of CR1 or intact E^{hu}, containing an equivalent amount of CR1. Following incubation at 30°C for 30 min, the percentage of release of C3 radiolabel was determined (Fig. 8). Release of C3 radiolabel increased in a dose-dependent fashion as the amount of human C4 added to the cells was increased, whether the release was supported by purified CR1 (Fig. 8*A*) or by membrane-associated CR1 on intact E^{hu} (panel B). Minimal release occurred in the presence of I alone, regardless of the C4 concentration used to prepare the cells.

Kinetics of C4 and C3 Fragmentation. Kinetic analyses were next performed to determine the temporal relationship between *C4b and *C3b breakdown and release. EAC1423 bearing 3×10^3 molecules of ^{125}I-*C4b and 2×10^4 molecules of ^{131}I-labeled *C3b (*C3b/*C4b molar ratio of 7:1), or EAC142 bearing the same number of ^{125}I-labeled *C4b molecules, were mixed with I (16 μg/ml) and CR1 (200 ng/ml). After incubation at 37°C for increasing times (2–64 min), the reactions were stopped and the percentages of released C4 and C3 labels were determined. As shown in Fig. 9, although the presence of *C3b accelerated the release of C4 label from EAC1423 (compared with that from EAC142), almost 60% of C3 label had already been released before 10% of C4 label was found in the supernatant.

Additional kinetic analyses were performed using cells bearing different *C3b/*C4b ratios. Release of C3 label preceded that of C4 label in all cases. At very high densities of bound *C3b (>10^5 molecules/cell) and *C3b/*C4b ratios of >50:1, the initial rate of release of C4 label from EAC1423 was less than that

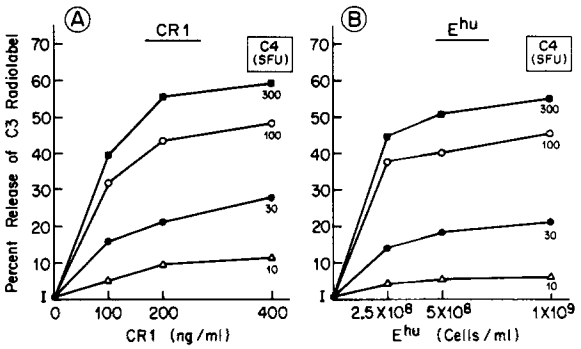

FIGURE 8. Enhancing effect of *C4b on the degradation of bound *iC3b by I plus CR1 or I plus E^{hu}. EAC1423 were prepared with limited human C4 (4 SFU) and sufficient ^{125}I-C3 to yield an uptake of 3.5×10^3 *C3b molecules per cell. The cells (5×10^7/ml) were then incubated at 30°C for 30 min with I (2 μg/ml) and H (10 μg/ml). Titrations revealed >90% loss of C3b hemolytic activity. The EAC141im3i (5×10^7/ml) were then reincubated at 30°C for 20 min with C1 (300 SFU) and then with 10, 30, 100, or 300 SFU of (human) C4. After washing, the cells (5×10^7/ml) bearing increasing amounts of *C4b and a constant amount of ^{125}I-*iC3b were incubated at 30°C for 30 min with I (2 μg/ml) plus increasing concentrations of CR1 or increasing numbers of E^{hu}. The percentage of release of C3 radiolabel is shown as a function of CR1 or E^{hu} input in *A* and *B*, respectively. Addition of *C4b enhanced the release of C3 label whether purified CR1 or E^{hu} were used.

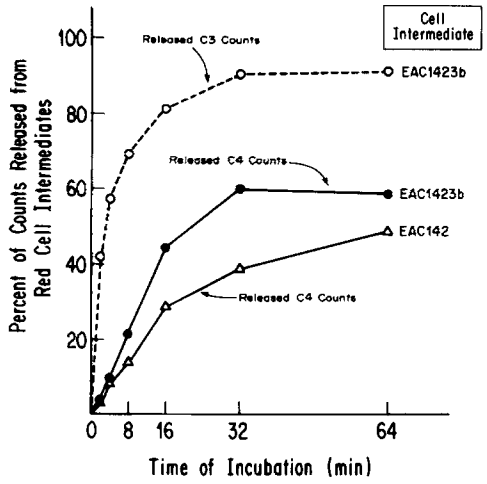

FIGURE 9. Kinetics of fragmentation of *C4b and *C3b by I plus CR1. EAC142 (5×10^7/ml) bearing 3×10^3 *C4b molecules and either 0 or 2×10^4 molecules of *C3b were incubated at 37°C with I (16 μg/ml) plus CR1 (200 ng/ml) and the reaction stopped after different times. The percentage of release of ^{125}I-C4 label from EAC142 and of ^{125}I-C4 and ^{131}I-C3 labels from EAC1423 are shown as a function of incubation time. The presence of *C3b accelerated the release of C4 label. Nevertheless, release of C3 label preceded release of C4 label.

from EAC142. Nevertheless, release of C4 label after 60 min at 37°C from EAC1423 in all cases studied exceeded that from EAC142 (not shown). Even when the *C3b/*C4b ratio was reversed and cells bearing low densities of *C3b (1×10^3 molecules/cell) and high densities of *C4b (8×10^3 molecules/cell) were used, release of C3 label still preceded that of C4 label.

*Analysis of the Products of I plus CR1-mediated Degradation of *C4b on Cells Bearing *C4b and *C3b.* Previous studies have shown that the C3b fragments generated by I plus CR1 are the same whether *C3b is deposited on the substrate by the classic (1) or by the alternative pathways (2, 3, 31), indicating that the presence of nearby *C4b molecules does not alter the pattern of *C3b breakdown. To determine whether the presence of neighboring *C3b molecules influences the pattern of *C4b breakdown, EAC14 and EAC1423 were prepared with ^{125}I-C4 and treated with I plus CR1, E^{hu}, or C4bp. The supernatants and stroma of each reaction mixture were isolated and structural analyses performed by SDS-PAGE and radioautography. As shown in Fig. 10, (lanes 1–3, 5, 7) I, CR1, E^{hu}, or C4bp alone had negligible activity.

The major fragments released from EAC14 or EAC1423 by I plus CR1, I plus E^{hu}, or I plus C4bp were identical. They had molecular weights of 140,000 (140 K) under nonreducing conditions, and after reduction (Fig. 10, lanes 4, 6, 8), they yielded peptides of 75 K and 31 K mol wt, corresponding to β and γ chains, and of ~25 K and 17 K mol wt, corresponding to α′3 and α′4 segments of α′ chain. The amount of released C4c was much smaller in the presence of I plus C4bp (lane 8).

The SDS-PAGE of the stroma of EAC1423, isolated after the various treatments (lanes 9–13) revealed the presence of remaining *C4b α′, β, and γ chains.

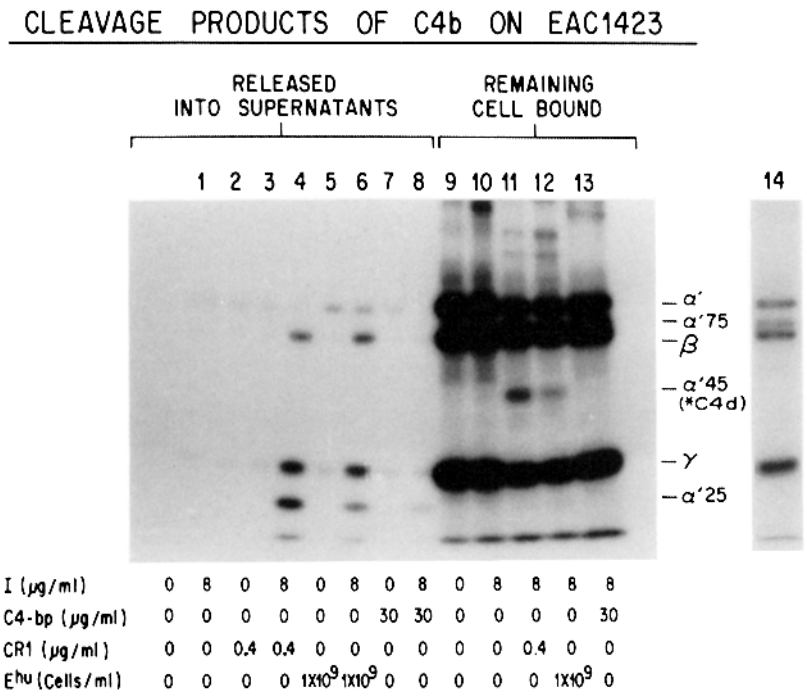

FIGURE 10. SDS-PAGE analysis of *C4b fragments generated by I plus CR1, E^{hu}, or C4bp in the presence of *C3b. EAC1423 (5×10^7/ml), bearing 3×10^5 molecules of ^{125}I-*C4b and 2×10^4 molecules of (unlabeled) *C3b, were incubated at 37°C for 60 min with $DGVB^{++}$, I alone (8 μg/ml), CR1 alone (400 ng/ml), E^{hu} alone (1×10^9/ml), C4bp alone in equivalent blood proportion (30 μg/ml), or with I plus CR1, I plus E^{hu}, or I plus C4bp at the same concentrations. Samples of supernatants and cell stroma in each reaction mixture were subjected to SDS-PAGE under reducing conditions and radioautographs prepared. A small amount of polypeptides with molecular weights corresponding to α', β, and γ chains of uncleaved C4b were released into the supernatant by $DGVB^{++}$, I, CR1, E^{hu}, or C4bp alone (lanes 1, 2, 3, 5, and 7 respectively). Polypeptides with molecular weights corresponding to β and γ chains, and α'3 and α'4 fragments, were released in small amounts in the presence of I alone (lane 2) or I plus C4bp (lane 8) but in much larger amounts in the presence of I plus CR1 or I plus E^{hu} (lanes 4 and 6). The *C4b polypeptides released by I plus CR1 or I plus E^{hu} were identical to those generated in the absence of *C3b (not shown).

Polypeptides corresponding to the α', β, and γ chains of uncleaved *C4b were seen in all samples of stroma (lanes 9–13). Additional polypeptides of 75 K mol wt were seen in small amounts in samples treated with I alone (lane 10) and in greater amounts in samples treated with I plus C4bp (lane 13). These quantitative differences are not clearly seen in the above radioautography because the α' 75 and β chain bands appear fused (the time of exposure was increased to show the α' fragments released in the supernatant). In lane 14 we show a repeat of the radioautography of lane 13 with less exposure time to permit the distinction between the α' 75 and β peptides.

Polypeptides of 45 K mol wt (probably resulting from further cleavage of the 75 K mol wt fragments) were prominent only in samples treated with I plus CR1 or I plus E^{hu} (lanes 11, 12). Identical peptides were observed in the absence of *C3b (not shown) except that small amounts of 45 K mol wt peptides were also seen in samples treated with I plus C4bp.

EAC1423 stroma treated with I and the different cofactors additionally yielded peptides of 45 K mol wt presumably representing covalently bound α'2 (*C4d) fragments, as well as peptides of 75 K mol wt, which appear fused with the β chain band. In lane 14 we show a repeat of the radioautography of lane 13 with

less exposure time, which permits the distinction between the $\alpha'75$ and β peptides.

A comparison of the patterns seen in samples derived from the stroma of cells treated with I plus C4bp with those in samples treated with I plus CR1 or I plus E^{hu} showed that they differed in that the proportion of the 45 K mol wt peptide relative to the 75 K mol wt peptide was much smaller in the samples treated with C4bp plus I (lane 13). The high molecular weight bands seen in lanes 9–13 probably represent α' chain fragments covalently bound to macromolecules from the cell membrane.

Discussion

The mechanisms regulating the function of *C4b have remained incompletely understood. Although C4bp is required for cleavage of fluid phase C4b by I, the α' chain of *C4b can be cleaved successively at two or more sites by I alone and C4c subsequently released from the substrate (19, 30). Relatively high concentrations of I and prolonged incubation are necessary, however, and degradation is usually incomplete. One study (29) shows that C4bp can enhance this reaction, while another (19) reports that C4bp has no effect. Recently, Fujita and Tamura (28) have resolved this controversy by showing that an enhancing action of C4bp can be demonstrated only at high *C4b density ($>3 \times 10^3$ molecules/cell).

Initial experiments of ours (1) indicated that CR1 also did not appear to substantially alter I-mediated release of C4c. These earlier studies were performed with low concentrations of I and CR1 and a temperature of incubation of 30°C; that is, under reaction conditions similar to those which we used to study degradation of *C3b. In the present paper we present the results of additional studies, performed under different experimental conditions. We show here that in the presence of higher concentrations of I, CR1 does promote the degradation of *C4b on EAC14 cells provided that the density of *C4b is $>3 \times 10^3$ molecules per cell, and that the incubation is carried out at either 37°C or alternatively for longer periods of time at 30°C. Under these conditions, on a weight basis, CR1 is $\sim 10^3$-fold more effective than C4bp in promoting fragmentation of *C4b on EAC14 (Fig. 2). Since it could be argued that the behavior of the detergent-extracted and purified CR1 in an aqueous environment might differ from that of membrane-associated CR1, these and other experiments (Figs. 2, 8, 10) were also performed using intact human erythrocytes as a source of receptor. The results showed that the CR1 activity on E^{hu} and in purified form was comparable. In addition, the experiments revealed that the cofactor activity of erythrocytes was almost exclusively mediated by CR1, since it was strongly inhibited by monoclonal antibodies to the receptor (Fig. 2).

Perhaps of greater significance is the finding that the breakdown of *C4b by I plus CR1 was enhanced by the presence of *C3b on the cellular intermediates (Fig. 3). On EAC1423 cells, CR1 supported the *C4b breakdown even at low *C4b densities and low concentrations of I and CR1, under incubation conditions comparable to those required for *C3b breakdown (1). It appears, therefore, that in the physiological situation, before *C3b uptake, *C4b is less vulnerable to attack by I. Once optimal *C3b uptake has taken place, binding to CR1 is

enhanced and the activities of the *C4b- and *C3b-dependent enzymes are inhibited.

Despite the marked difference in efficiency between CR1 and C4bp in promoting I-mediated *C4b fragmentation, similar degradation products were obtained from EAC14 or EAC1423 cells (Fig. 10). The C4c fragment released into the supernatant, after incubation with I and either cofactor, had a molecular weight of 140 K and consisted of polypeptides of 75 K, 31 K, 25 K, and 17 K mol wt, corresponding to β and γ chains and $\alpha'3$ and $\alpha'4$ segments, respectively. The bands remaining associated with the cell had molecular weights of about 75 K and 45 K, the latter most likely representing *C4d (28, 30). As shown by Fujita and Tamura (28), the 75 K band derives from an intermediate product of degradation of C4b (iC4b) first described by Nagasawa et al. (32). The results of multiple experiments, one of them represented in Fig. 10, showed that the 45 K/75K ratio was much higher in *C4b samples treated with I plus CR1 than in those treated with I plus C4bp, suggesting that the cleavage of *iC4b by I is promoted by CR1 more effectively than by C4bp. These findings indicate, therefore, that the efficiency of the I-mediated conversion of *C4b into C4c and *C4d is substantially greater in the presence of CR1 than of C4bp. This difference in the effects between I plus CR1 and I plus C4bp parallels the differential effects of I plus CR1 and I plus H on *C3b (1, 4).

In analogy to the above findings showing that *C3b enhances degradation of *C4b, studies with cells bearing limited amounts of *C3b demonstrated a parallel potentiating effect of *C4b on *C3b degradation. That this effect was indeed mediated by *C4b was shown by several observations. For example, binding of C2a or anti-C4 antibody to *C4b on EAC14231im inhibited the I plus CR1–mediated C3c release (Fig. 7). Reduction of *C4b density on the cells or replacement of human *C4b by guinea pig *C4b (which most likely has a lower binding affinity for human CR1) had a similar effect (Fig. 6). Uptake of additional *C4b onto EAC143i increased I plus CR1–mediated C3c release in a dose-dependent fashion (Fig. 8). The most plausible mechanism of these effects is that *C4b promotes interaction of *C3b or *iC3b with CR1.

Provided that the kinetics of C3c and C4c release from EAC1423 treated with CR1 plus I (Fig. 9) parallel the kinetics of the underlying proteolysis of the α' chains of *C3b and *C4b, the enzymatic attack on *C3b proceeds faster than that on *C4b. The simplest explanation for this observation is that CR1 has greater affinity for *C3b than for *C4b. It is also conceivable that the spatial arrangement of *C3b and *C4b on the substrate could influence the outcome of the reaction if multiple bonds have to be formed between CR1 and the substrate-bound ligands before the inactivator I can fully exert its effect.

The finding that *C4b can enhance *C3b interaction with CR1, taken together with the results above that the converse is also true, argue that clusters of *C4b*3b can serve as a ligand for CR1, which is itself probably multivalent (33, 34). This notion is consistent with previous observations that *C3b can enhance the rosetting of EAC14 with E^{hu}, and that excess *C4b can enhance rosetting of EAC14231im (18). A greater ability of *C4b*3b clusters than of *C4b or *C3b monomers alone to interact with CR1 is also compatible with more recent findings (35) that C3b dimer binds to CR1 with high affinity, while C3b monomer

does not. The formation of multiple bonds between ligand and CR1 can likewise account for the observation (4) that the activity of CR1 in the inactivation of classical pathway C5-convertase can be much greater than that of H, and that CR1 activity can increase progressively with increasing *C3b density on the substrate. This latter result, in conjunction with longstanding observations by many investigators that rosetting between EAC14 and E^{hu} occurs only at very high *C4b densities, and the multiple data showing cooperation between *C4b and *C3b in the present study, suggests that effective interaction with CR1 requires a multivalent ligand which can consist of clusters of *C4b, *C3b, *iC3b, *iC4b, or of these fragments, in various combinations.

In summary, the present results strongly suggest that *C4b is more effectively inactivated when *C3b is fixed, and that the inactivation of *C4b*3b by I is greatly enhanced by CR1, but not by C4bp. The greater ability of CR1 than C4bp to serve as a cofactor of I in this reaction can be explained by the lack of high binding affinity of C4bp for *C3b (30), but steric considerations arising from the multimeric nature of the interaction between substrate and cofactor may also be important. The findings of this paper also have implications in the pathophysiology of complement deficiency diseases. For example, they predict that in C2-deficient individuals, *C4b should persist and thereby accumulate to a greater extent on substrates, since in these individuals *C3b cannot be deposited by the classical pathway and *C4b inactivation therefore should be less efficient. On the other hand, when CR1 levels on erythrocytes are diminished, as, for example, can occur in patients with systemic lupus erythematosus (11–15), *C4b and *C4b*3b clusters will be less subject to I-mediated control and complement consumption by the classical pathway will increase.

Summary

The complement fragments C3b and C4b are the main ligands for the membrane receptor CR1. We showed elsewhere that CR1 functions as an essential cofactor for the factor I–mediated enzymatic breakdown of membrane-bound C3b (*C3b) into C3c and *C3dg. One of the main findings of the present paper is that CR1 also promotes the degradation of bound C4b (*C4b) into C4c and *C4d. On a weight basis, the cofactor activity of CR1 in the cleavage of *C4b present on the cell intermediate EAC14 is 10^3-fold greater than that of the serum cofactor C4-binding protein (C4bp).

An additional finding is that the effect of CR1 on either *C3b or *C4b is modulated by the presence of the other ligand in its vicinity; that is, *C4b degradation by CR1 plus I is enhanced by neighboring *C3b and vice versa. For example, upon uptake of optimal amounts of *C3b onto EAC142 and the assembly of the C3-convertase EAC1423, the activity of CR1 in generating C4c is enhanced 5–10 times further. Conversely, when the number of *C3b molecules on EAC1423 is relatively small (or when EAC1423 has been converted by I plus H into EAC1423i), the presence of neighboring *C4b enhances the conversion of *C3b (or *iC3b) into C3c plus *C3dg.

The enhancing effect of *C3b on the cleavage of *C4b by I is observed only if the cofactor of this reaction is CR1. Indeed, the activity of I or I plus C4bp on *C4b is significantly inhibited when *C3b is fixed and the main product of the

reaction is *iC4b. Taken together, these findings suggest that degradation of *C4b will be more effective when enough C3b molecules are fixed nearby, thus facilitating the interaction of *C4b*3b clusters with CR1-bearing cells, and that under physiological conditions, *C4b activity can be efficiently controlled by CR1.

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References

1. Medof, M. E., K. Iida, C. Mold, and V. Nussenzweig. 1982. Unique role of the complement receptor CR1 in the degradation of C3b associated with immune complexes. *J. Exp. Med.* 156:1739.
2. Ross, G. D., J. D. Lambris, J. A. Cain, and S. L. Newman. 1982. Generation of three different fragments of bound C3 with purified factor I or serum. I. Requirements for factor H vs. CR1 cofactor activity. *J. Immunol.* 129:2051.
3. Medicus, R. G., J. Melamed, and M. A. Arnaout. 1983. Role of human factor I and C3b receptor in the cleavage of surface-bound C3bi molecules. *Eur. J. Immunol.* 13:465.
4. Medof, M. E., and V. Nussenzweig. 1983. Differential activities of CR1 and H in regulation of the function of substrate-bound and fluid-phase C3b. *Immunobiology.* 164:275. (Abstr.)
5. Fearon, D. T. 1979. Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane. *Proc. Natl. Acad. Sci. USA.* 76:5867.
6. Medof, M. E., and Oger, J. J.-F. 1982. Competition for immune complexes by red cells in human blood. *J. Clin. Lab. Immunol.* 7:7.
7. Medof, M. E., and G. M. Prince. 1983. Alternative pathway-mediated rebinding of immune complexes to human red blood cells. *Immunology.* 50:1.
8. Medof, M. E., and G. M. Prince. 1983. Immune complex alterations occur on the human red blood cell membrane. *Immunology.* 50:11.
9. Cornacoff, J. B., L. A. Hebert, W. L. Smead, M. E. VanAman, D. J. Birmingham, and F. J. Waxman. 1983. Primate erythrocyte-immune complex-clearing mechanism. *J. Clin. Invest.* 71:236.
10. Siegel, I., and N. Gleicher. 1983. The red-cell immune system. The red army in the war against disease: white cells may not stand alone. *Science (Wash. DC).* 23(1):30.
11. Miyakawa, Y., A. Yamada, K. Kosaka, F. Tsuda, E. Kosugi, and M. Mayumi. 1981. Defective immune adherence (C3b) receptor on erythrocytes from patients with systemic lupus erythematosus. *Lancet.* II:493.
12. Iida, K., R. Mornaghi, and V. Nussenzweig. 1982. Complement receptor (CR1) deficiency in erythrocytes from patients with systemic lupus erythematosus. *J. Exp. Med.* 155:1427.
13. Wilson, J. G., W. W. Wong, P. H. Schur, and D. T. Fearon. 1982. Mode of inheritance of decreased C3b receptors on erythrocytes of patients with systemic lupus erythematosus. *N. Engl. J. Med.* 307:981.
14. Kazatchkine, M. D., D. T. Fearon, M. D. Appay, C. Mandet, and J. Bariety. 1982. Immunohistochemical study of the human glomerular C3b receptor in normal kidney

and in seventy-five cases of renal diseases: loss of C3b receptor antigen in focal hyalinosis and in proliferative nephritis of systemic lupus erythematosus. *J. Clin. Invest.* 69:900.

15. Taylor, R. P., C. Horgan, R. Buschbacher, C. M. Brunner, C. E. Hess, W. M. O'Brien, and H. J. Wanebo. 1983. Decreased complement-mediated binding of antibody/^{3}H--dsDNA immune complexes to the red blood cells of patients with systemic lupus erythematosus, rheumatoid arthritis, and hematologic malignancies. *Arthritis Rheum.* 26:736.
16. Goers, J. W. F., and R. R. Porter. 1978. The assembly of early components of complement on antibody-antigen aggregates and on antibody-coated erythrocytes. *Biochem. J.* 175:675.
17. Cooper, N. R. 1969. Immune adherence by the fourth component of complement. *Science (Wash. DC).* 165:396.
18. Bokish, V. A., and A. T. Sobel. 1974. Receptor for the fourth component of complement on human B lymphocytes and cultured human lymphoblastoid cells. *J. Exp. Med.* 140:1336.
19. Iida, K., and V. Nussenzweig. 1980. Complement receptor is an inhibitor of the complement cascade. *J. Exp. Med.* 153:1138.
20. Tack, B. F., J. Janotova, M. L. Thomas, R. A. Harrison, and C. H. Hammer. 1980. Isolation and properties of complement components. *Methods Enzymol.* 80:64.
21. Kerr, M. A. 1980. The second component of human complement. *Methods Enzymol.* 80:54.
22. Polley, M. J., and H. J. Muller-Eberhard. 1967. Enhancement of the hemolytic activity of the second component of complement by oxidation. *J. Exp. Med.* 126:1013.
23. Nelson, R. A., J. Jense, I. Gigli, and N. Tamura. 1966. Methods of separation, purification and measurement of nine components of hemolytic complement in guinea pig serum. *Immunochemistry.* 3:111.
24. Cooper, N. R., and H. J. Muller-Eberhard. 1970. The reaction mechanism of C5 in immune hemolysis. *J. Exp. Med.* 132:775.
25. Crossley, L. G., and R. R. Porter. 1980. Purification of the human complement control protein C3b inactivator. *Biochem. J.* 191:173.
26. Nussenzweig, V., and R. Melton. 1980. Human C4-binding protein (C4-bp). *Methods Enzymol.* 80:124.
27. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680.
28. Fujita, T., and N. Tamura. 1983. Interaction of C4-binding protein with cell bound C4b: a quantitative analysis of binding and the role of C4-binding protein in proteolysis of cell-bound C4b. *J. Exp. Med.* 157:1239.
29. Gigli, I., T. Fujita, and V. Nussenzweig. 1979. Modulation of the classical pathway C3 convertase by plasma proteins C4 binding protein and C3b inactivator. *Proc. Natl. Acad. Sci. USA.* 76:6596.
30. Fujita, T., and V. Nussenzweig. 1979. The role of C4-binding protein and β1H in proteolysis of C4b and C3b. *J. Exp. Med.* 150:267.
31. Mold, C., and M. E. Medof. 1983. C3 nephritic factor protection of bound C3 from breakdown by C3b/C4b inactivator and the complement receptor CR1. *Immunobiology.* 164:278. (Abstr.)
32. Nagasawa, S., C. Ichihara, and R. M. Stroud. 1980. Cleavage of C4b by C3b inactivator:production of a nicked form of C4b, C4b′, as an intermediate cleavage product of C4b by C3b inactivator. *J. Immunol.* 125:578.
33. Dierich, M. P., and R. A. Reisfeld. 1975. C3-mediated cytoadherence. Formation of C3 receptor aggregates as prerequisite for cell attachment. *J. Exp. Med.* 142:242.

34. Abrahamson, D. R., and D. T. Fearon. 1983. Endocytosis of the C3b receptor of complement within coated pits in human polymorphonuclear leukocytes and monocytes. *Lab. Invest.* 48:162.
35. Arnaout, M. A., J. Melamed, B. F. Tack, and H. R. Colten. 1981. Characterization of the human complement (C3b) receptor with a fluid phase C3b dimer. *J. Immunol.* 127:1348.
36. Arnaout, M. A., N. Dana, J. Melamed, R. Medicus, and H. R. Colten. 1983. Low ionic strength or chemical cross-linking of monomeric C3b increases its binding affinity to the human complement C3b receptor. *Immunology.* 48:229.