COMPLEMENT RECEPTOR IS AN INHIBITOR OF THE COMPLEMENT CASCADE*

By KYOKO IIDA AND VICTOR NUSSENZWEIG

From the Department of Pathology, New York University Medical Center, New York 10016

Human erythrocytes interact with particles or soluble immune complexes bearing C3b, the major fragment of the third component of complement. This phenomenon is known as immune adherence (1). B lymphocytes (2), neutrophils, macrophages, monocytes (3–5), and epithelial cells of the kidney glomerulus (6) also have membrane receptors for C3b. The C3b receptors function in phagocytosis, facilitating the attachment of the particle to the phagocytes, and, in synergy with the Fc receptors for IgG, promote ingestion of sensitized particles (7, 8). Recently, a glycoprotein of 205,000 mol wt has been isolated from the membrane of human erythrocytes and identified as the C3b receptor (CR1) (9, 10). This protein was characterized as an inhibitor of the alternative pathway C3 convertase (C3b,Bb) and as a cofactor for the cleavage of the α' chain of C3b in the fluid phase by a serum enzyme, C3b/C4b inactivator.

Complement receptors for C3b have also been reported to bind C4b (11, 12). For this reason, we searched for evidence of interactions between C4b and CR1 by studying the effects of CR1 on the enzymes of the classical complement pathway whose functions are C4b dependent; that is, C4b,2a and C4b,2a,3b (C3 and C5 convertases).

Materials and Methods

Buffers. Buffers used were: isotonic veronal-buffered saline with 2.5% dextrose containing 0.1% gelatin, 0.5 mM $MgCl_2$, and 0.15 mM $CaCl_2$ (DGVB);[1] veronal-buffered saline containing 0.1% gelatin and 0.01 M EDTA (EDTA-GVB); and veronal buffer with 2.5% dextrose containing 0.1% gelatin and 0.01 M EDTA (EDTA-DGVB; 13).

Complement Components. Human (hu) C1s (14), C4 (15), C3 (16), C4 binding protein (C4-bp; 17), C3bINA (18), factor B (19), and properdin (P; 20) were purified as previously described. C2 was purified according to Nagasawa et al. (21), except that the final step (affinity chromatography on Sepharose C4) was omitted. C2 was oxidized as described (22) and referred to as ^{oxy}C2. C5 was purchased from Cordis Laboratories, Inc., Miami, Fla. Guinea pig (gp) C1 was partially purified as described in (23). C6-9 was prepared from guinea pig serum (24).

* Supported by grants AI 08499 and CA 16247 from the National Institutes of Health, Bethesda, Md.

[1] *Abbreviations used in this paper:* B, factor B; ^{oxy}C2, oxidized human C2; C4-bp, C4 binding protein; CR1, complement receptor for C3b and C4b (immune adherence receptor); DGVB, veronal-buffered saline with 2.5% dextrose containing 0.1% gelatin, 0.5 mM $MgCl_2$, and 0.15 mM $CaCl_2$; EA, sheep erythrocytes sensitized with rabbit IgG antibody; EAC1, EAC14, EAC14^{oxy}2, and EAC14^{oxy}23, cellular intermediates prepared with EA and bearing the major cleavage fragments of the designated complement component; EAC14$_{lim}$, EAC142$_{lim}$, EAC14^{oxy}2$_{lim}$, EAC14^{oxy}23$_{lim}$, and EAC14^{oxy}235$_{lim}$, cellular intermediates, same as above, except that the final components of each were in limiting amounts; EDTA-DGVB, veronal buffer with 2.5% dextrose containing 0.1% gelatin and 0.01 M EDTA; EDTA-GVB, veronal-buffered saline containing 0.1% gelatin and 0.01 M EDTA; gp, guinea pig; hu, human; NP-40, Nonidet P-40; P, properdin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SFU, site-forming units.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/81/05/1138/13 $1.00
Volume 153 May 1981 1138–1150

Cellular Intermediates. EAC1, EAC14, EAC14^{oxy}2, and EAC14^{oxy}23 were prepared from antibody (IgG)-sensitized sheep erythrocytes (EA) by reacting sequentially with 300 site-forming units (SFU) of C1gp, C4hu, ^{oxy}C2hu, and C3hu until the corresponding stage of each of the intermediate cells was reached. EAC14 cells bearing 1.0–1.5 C4 sites/cell were prepared by incubating EA with excess C1 (300 SFU) and limiting amounts of C4hu. The cells were referred to as EAC14$_{lim}$, EAC142$_{lim}$, EAC14^{oxy}2$_{lim}$, EAC14^{oxy}23$_{lim}$, and EAC^{oxy}235$_{lim}$, and were prepared in the same manner, except that the limiting components were the final ones in each intermediate.

Complement Receptor. CR1 was purified by sequential column chromatography on Biorex-70 (Bio-Rad Laboratories, Richmond, Calif.), Sepharose-lentil lectin, and Sepharose-C3 affinity chromatography, according to the method of Fearon (9). During purification, CR1 was assayed by its inhibitory activity for C3 convertase either of the classical (C4b,2a) or the alternative (C3b,Bb,P) pathway. Inhibition of the alternative pathway C3 convertase was assayed as previously described (9). Inhibition of the classical pathway C3 convertase was assayed as follows: 100 μl of EAC142$_{lim}$, 1 × 10^8/ml, was incubated with 100 μl of samples from fractions diluted in DGVB for 15 min at 30°C. Residual C4b,2a activity was developed by adding 1.3 ml of guinea pig serum diluted 1:50 with EDTA-GVB, followed by incubation at 37°C for 60 min. The protein concentration of CR1 was estimated by a modified Folin method (25) using bovine serum albumin (BSA) as a standard.

Radiolabeling. C4 was labeled with ^{125}I by means of Enzymobeads (Bio-Rad Laboratories). 25 μg of C4 in 25 μl was incubated with 50 μl of Enzymobeads, 1 mCi of ^{125}I (Amersham Corp., Arlington Heights, Ill.), and 25 μl of 2% glucose. After incubation for 20 min at 0°C, the reaction was stopped by addition of 50 μl of 0.05% sodium azide. Free iodine was removed by dialysis. The specific activity was 4 × 10^6 cpm/1 μg protein. ^{125}I-C4b was prepared by incubation of 25 μg ^{125}I-C4 with 1 μg of C1s at 37°C for 2 h.

CR1 was labeled by the same method used for C4, and separated from the free iodine as follows: the labeled mixture was diluted with 0.1% Nonidet P-40 (NP-40; Particle Data, Inc., Elmhurst, Ill.) and applied to a 0.3-ml Biorex-70 column equilibrated with 0.05 M phosphate buffer, pH 7.2, containing 0.02 M NaCl and 0.1% NP-40. The column was washed extensively in the starting buffer and ^{125}I-CR1 was eluted with 0.5 M NaCl. The specific activity was 7 × 10^4 cpm/1 μg protein.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). This was performed according to the method of Laemmli (26). 10% running and 5% stacking gels were used. For labeled samples, radioautography was carried out by exposing the dried gel to an X-Omat R film (XR-5; Eastman Kodak Company, Rochester, N. Y.) at −70°C overnight. For estimation of the molecular weight of the bands, the following standards were used: 200,000 myosin (H chain); 92,500 phosphorylase B; 68,000 BSA; 43,000 ovalbumin; 25,700 α-chymotrypsinogen; and 18,400 β-lactoglobulin.

Results

Purification of an Inhibitor of C4b,2a from Human Erythrocyte Membranes. The inhibitory factor was isolated from human erythrocytes as described by Fearon (9), except that the membranes originating from 2 × 10^{13} erythrocytes were washed extensively in a Pellicon Cassette System (Millipore Corp., Bedford, Mass.), and concentrated to a volume of 300 ml before solubilization in detergent and loading in a Biorex-70 column. The activity of the inhibitor was followed by two assays; that is, the decay-dissociation of C4b,2a and C3b,Bb,P formed on sheep erythrocytes. During all steps of purification, the two activities coincided as illustrated in Fig. 1. 30 μl of the purified inhibitor at a concentration of 200 μg/ml gave a single stained band in SDS-PAGE under reducing or nonreducing conditions, with a molecular weight close to 200,000. Therefore, a single glycoprotein from human erythrocyte membranes, identified as the complement receptor (CR1) of cells (10), inhibits the activity of the C3 convertase of both the classical and alternative pathways.

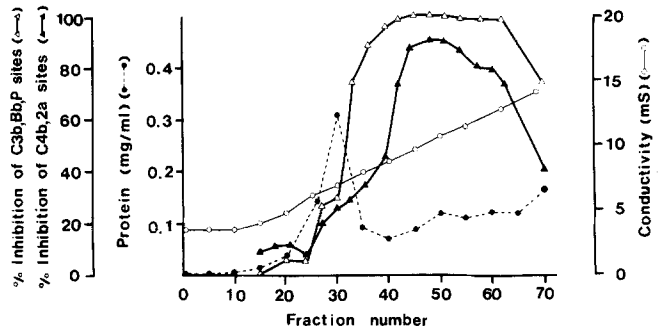

FIG. 1. Chromatography on Biorex-70 of extracts of erythrocyte membranes. 2×10^{13} human erythrocytes were washed in phosphate-buffered saline (0.15 M, pH 7.4) and lysed in 18 liters of hypotonic buffer (5 mM phosphate, 0.5 mM EDTA). The membranes were washed extensively with the same hypotonic buffer containing 0.5 mM phenyl methyl-sulfonyl fluoride, by passage through a Pellicon Cassette System and centrifuged at 18,000 *g* for 1 h. To the collected membranes in a volume of about 300 ml, an equal volume of 1% NP-40 in 5 mM phosphate buffer containing 0.15 M sodium chloride and 0.5 mM EDTA was added and incubated for 3 h in the cold. After solubilization, 1,200 ml of 0.05 M phosphate buffer, pH 7.2, was added to adjust the conductivity to 3.8 mS (0°C) and the insoluble fraction was removed by centrifugation. The supernate was loaded in a Biorex-70 column (3 × 15 cm) equilibrated with 0.05 M phosphate buffer containing 0.1% NP-40 and 0.02 M sodium chloride, pH 7.2, (conductivity 3.8 mS). The column was washed with 1 liter of the same buffer and a linear salt gradient was applied. The gradient was formed by mixing 400 ml of the starting buffer with 400 ml of this buffer containing 0.35 M sodium chloride. The fractions were assayed as follows: samples were diluted 1:20 and 100 μl were incubated for 15 min at 30°C with equal volumes of $EAC142_{lim}$ or EAC43BP (1×10^8/ml). Then, 1.3 ml of guinea pig serum diluted 1:50 with EDTA-GVB or 1:20 with EDTA-DGVB was added to $EAC142_{lim}$ or EAC43BP, respectively. The tubes were incubated at 37°C for 1 h and the degree of lysis was measured. It is clear that the two inhibitory activities were eluted from the column in a parallel fashion. The inset (middle track) shows the results of the 4.5% SDS-PAGE, under nonreducing conditions, of the final purified CR1 obtained after additional steps of purification. Also shown are purified β1H (left track) and C4 (right track).

Mechanism of Inhibition of the Classical Pathway C3 Convertase. The inhibitory activity of CR1 on C3 consumption by C4b,2a was directly shown as follows: $EAC14^{oxy}2$ cells were prepared with 300 SFU of C1gp, C4hu, and ^{oxy}C2hu. Samples (0.2 ml, 2×10^7 cells) were incubated with equal volumes of various dilutions of CR1 in DGVB for 30 min at 30°C. The cells were washed twice by centrifugation, the pellets were incubated with 0.2 ml of purified human C3 (36 SFU) for 30 min at 30°C, and the residual C3 hemolytic activity was determined. The results showed a dose-dependent inhibition of C3 consumption by CR1 (Fig. 2). The effects could be observed at concentrations of the receptor <50 ng/ml.

The experiments illustrated in Fig. 3 A demonstrate that this inhibitory activity is associated with the decay of C4b,2a sites from the EAC142 cells. Erythrocyte intermediates were prepared with 300 SFU of C1 and C4, but with limiting amounts of C2. The $EAC142_{lim}$ cells were incubated with equal volumes of various dilutions of CR1 at 30°C and the time course of the C4b,2a decay was studied. The half-life of the enzyme was considerably shortened in the presence of the receptors in a dose-related manner. For example, at a concentration of CR1 of 95 ng/ml, the half-life of the C4b,2a sites was 4.5 min, whereas in controls incubated with buffer, the half-life was 8 min. Stabilized enzyme $C4b^{oxy}2a$ was also inhibited (Fig. 3 B).

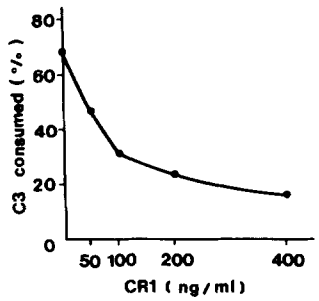

FIG. 2. Inactivation of C3-convertase activity by CR1. 0.2 ml of EAC14^{oxy}2 cells (1×10^8/ml) was incubated with an equal volume of CR1 for 30 min at 30°C and washed twice with DGVB. 0.2 ml of purified human C3 was added to each pellet. After 30 min of incubation at 30°C, supernatant fluids were separated and the residual C3 hemolytic activities were determined. The amount of C3 initially available (36 SFU) was calculated from the samples incubated with EAC14.

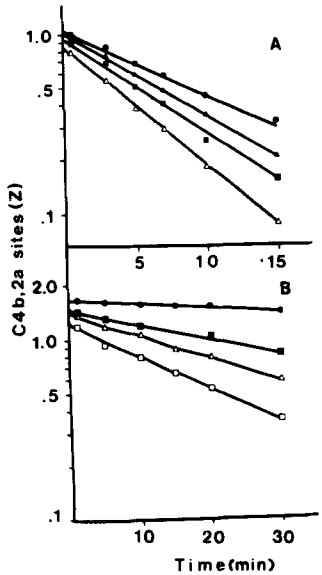

FIG. 3. Decay of C4b,2a sites from EAC142 cells prepared with C2 (A), or oxidized C2 (B). 1 ml of EAC142$_{lim}$ (1×10^8/ml) or EAC14^{oxy}2$_{lim}$ was mixed with an equal volume of pre-warmed DGVB (●), or with CR1 at final concentrations of 24 (▲), 48 (■), 95 (△) and 190 (□) ng/ml, and incubated at 30°C. At timed intervals, 200-μl samples were withdrawn, and 1.3 ml of guinea pig serum diluted 1:50 with EDTA-GVB was added. The mixtures were incubated for 60 min at 37°C to develop lysis. In the experiment using ^{oxy}C2, samples were washed twice after incubation with CR1.

Because C4-bp and CR1 have similar effects on the classical pathway C3 convertase (27), we compared their activities and found that CR1 was the superior inhibitor (Fig. 4). On a weight basis, ~5–10 times less CR1 than C4-bp was necessary to inactivate the same number of C4b,2a sites. Due to nonspecific aggregation, micelle formation, or assembly of polymers, the apparent molecular weight of either protein by gel filtration under nondenaturing conditions is $\sim 1 \times 10^6$.

Because the immune adherence receptor binds C4b (11, 12), the most likely explanation for the inhibitory activity of CR1 on C4b,2a is that CR1 competes with C2 for a binding site on C4b. This was investigated by radiolabeling CR1 and

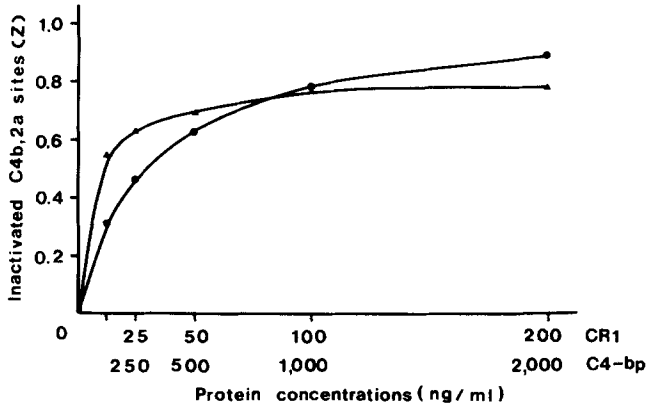

FIG. 4. Dose response curves of inactivation of C4b,2a by CR1 and C4-bp. 100 μl of $EAC14^{oxy}2_{lim}$ cells (1×10^8/ml) was incubated with 100 μl of serially diluted CR1 (▲), or C4-bp (●) at 30°C. After incubation for 30 min, cells were washed twice with DGVB, and then 1.5 ml of guinea pig serum diluted 1:50 with EDTA-GVB was added. The mixtures were incubated for hemolysis for 60 min at 37°C. The residual C4b,2a sites were then calculated. The number of C4b,2a sites in controls consisting of $EAC14^{oxy}2_{lim}$ cells incubated with DGVB was 0.99. It is apparent that CR1 is more effective than C4-bp in the inactivation of C4b,2a sites.

studying its incorporation onto EAC14 in the presence or absence of C2. In preliminary experiments, in which various erythrocyte intermediates were incubated with ^{125}I-radiolabeled CR1, we found that a maximum of 20% of the counts could be incorporated on EAC14, whereas only 3% bound EAC1 (data not shown).[2] Next, we studied the inhibitory effects of C2. Samples of $EAC14^{oxy}2$ cells were prepared with serially diluted ^{oxy}C2 and incubated with 100 μl of ^{125}I-CR1 (0.14 μg protein) at 30°C for 30 min. After washing, the binding of CR1 to cells was determined. Fig. 5 shows that ^{oxy}C2 prevented the binding of ^{125}I-CR1 to cells in a dose-dependent fashion. As a control, we found that purified factor B had no effect on the incorporation of ^{125}I-CR1 on EAC14 cells.

In all experiments so far described, the activity of CR1 on C4b,2a was studied on erythrocyte intermediates prepared with excess C4 (300 SFU). Under these conditions, it might be difficult to detect any direct and irreversible effects of CR1 on C4b. To investigate this possibility, we incubated samples of $EAC14_{lim}$ (0.83 sites/cell) with CR1 alone, or with CR1 plus C3b/C4b inactivator, or with buffer alone for 30 min at 30°C and measured the remaining hemolytic activity. As positive controls, other samples of $EAC14_{lim}$ were incubated with C3b/C4b inactivator, C4-bp, or mixtures of these two proteins. As shown in Fig. 6, CR1 and C4-bp alone did not influence the hemolytic activity of $EAC14_{lim}$ even at concentrations of 1.5 and 15 μg/ml, respectively. Furthermore, neither protein enhanced the enzymatic activity of C3b/C4b inactivator on the EAC14 cells.[3] This is in sharp contrast to the results of previous experiments showing that at concentrations 10 times smaller, CR1 inactivated 0.8 C4b,2a sites per $EAC142_{lim}$ cell (Fig. 4).

[2] About 43% of the counts bound to EA1423, probably reflecting a higher affinity of CR1 for C3b than for C4b.

[3] A previous report from our laboratory indicated that C4-bp enhances the cleavage of C4b by C3b/C4b inactivator (27). This conclusion was based mainly on the observation that C4-bp and C3b/C4b inactivator had synergistic inhibitory effects on the hemolytic activity of $EAC142_{lim}$ (rather than on $EAC14_{lim}$, which was used in the present experiments). In light of the results shown in Fig. 4, the pronounced diminution of C4b,2a sites on $EAC142_{lim}$ treated with C4-bp plus C3b/C4b inactivator, which we previously observed, could reflect an increased displacement of C2 by the combined activities of C4-bp and enzyme. This would not be without precedent, because C3b/C4b inactivator binds stoichiometrically to C3b in the presence of β1H (28).

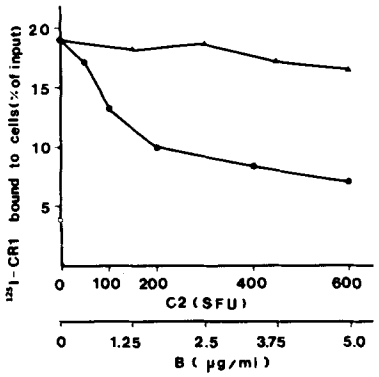

FIG. 5. Inhibition of ^{125}I-CR1 binding to cells by increasing amounts of ^{oxy}C2 (●), or factor B (▲). To 200 μl of EAC14 cells (1.5×10^8/ml), equal volumes of serially diluted oxidized C2 or factor B were added and incubated at 30°C. After 10 min, cells were washed and resuspended in 100 μl of DGVB. To each tube, 100 μl of ^{125}I-CR1 (1×10^4 cpm) was added. After incubation at 30°C for 30 min and addition of 1.3 ml of DGVB, the tubes were centrifuged. The cell pellets were washed once with DGVB. The percent of counts incorporated onto cells was determined by counting pellets and supernatant fluids. The binding of EAC1 was 3.9% (○). ^{oxy}C2, but not factor B, inhibited the incorporation of ^{125}I-CR1, in a dose-dependent fashion.

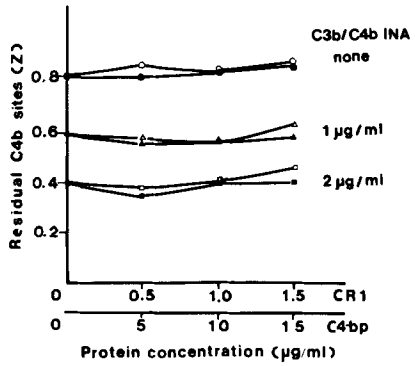

FIG. 6. Failure of CR1 and C4-bp to enhance the inactivation of the hemolytic activity of $EAC14_{lim}$ by C3b/C4b inactivator. Several aliquots of $EAC14_{lim}$ (1×10^8/ml) bearing 0.83 sites/cell were added to 100-μl mixtures of C3b/C4b inactivator, C4-bp, and CR1, and incubated at 30°C for 30 min. Then the cells were washed twice and the residual hemolytically active sites were developed by incubation with 300 SFU of C2gp at 30°C for 20 min, followed by further incubation with guinea pig serum diluted 1:50 in EDTA-GVB. Some of the experiments were performed in the absence of C3b/C4b inactivator (top two horizontal lines), or in the presence of 1 μg/ml (middle lines) or 2 μg/ml (bottom lines) of the enzyme. The results clearly show that increasing doses of CR1 (○, △, □) or of C4-bp (●, ▲, ■) were without effect in the inactivation of the hemolytic activity of $EAC14_{lim}$.

Taken together, these observations support the idea that CR1 and C2a compete for the same or similar sites on C4b, and that this may be the only basis for the inhibitory effects of CR1 on the classical pathway C3 convertase.

Inhibition of Classical Pathway C5 Convertase. The effect of CR1 on the classical pathway C5 convertase was investigated by measuring the hemolytic activity of $EAC14^{oxy}23_{lim}$ (100 μl, 10^7 cells) after incubation with serially diluted CR1 for 30 min at 30°C. The results are shown in Fig. 7. In the presence of 10 ng/ml of CR1, 50% of the available sites of C5 convertase were inhibited; that is, about 0.6 sites/cell. This

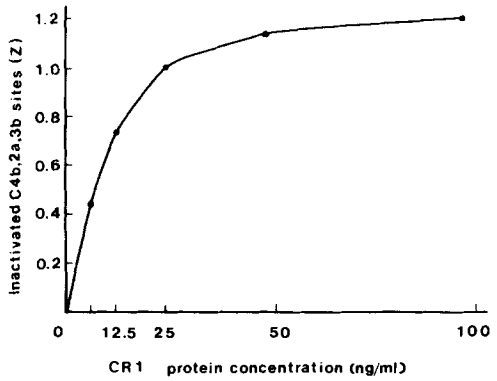

FIG. 7. Inactivation of C4b,2a,3b by CR1. 100 μl of $EAC14^{oxy}23_{lim}$ cells (1×10^8/ml) was incubated with 100 μl of serially diluted CR1 at 30°C for 30 min. Then 100 μl of C5hu was added at a dilution calculated to provide 300 SFU/cell, and the mixture was incubated at 30°C for 6 min. To develop lysis, 1.2 ml of the C6-9 reagent diluted 1:200 with EDTA-DGVB was added and incubated for 60 min at 37°C. The number of C4b,2a,3b sites available was 1.23, as determined in controls incubated in the absence of CR1.

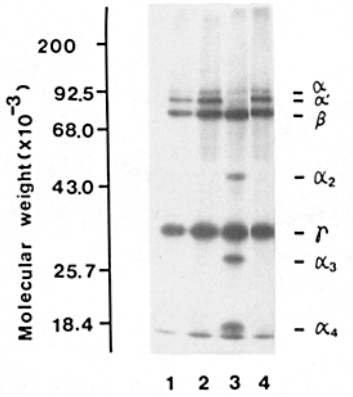

FIG. 8. Cleavage of C4b by CR1 and C3b/C4b inactivator. 0.04 μg of ^{125}I-labeled C4b was incubated with PBS alone (track 1), with 1.4 μg of CR1 (track 2), with 1.4 μg of CR1 and 0.16 μg of C3b/C4b inactivator (track 3), or with 0.16 μg of C3b/C4b inactivator (track 4) in a total volume of 30 μl at 37°C for 3 h. After reduction with 2-mercaptoethanol, samples were analyzed by 10% SDS-PAGE, followed by radioautography. CR1 functioned as an essential cofactor for the proteolysis of C4b by C3b/C4b inactivator. The α′ chain of C4b is cleaved into fragments α2, α3, and α4, in mixtures containing both CR1 and C3b/C4b inactivator, but it is not affected in the presence of either protein alone.

concentration of CR1 is three times smaller than that necessary to inactivate the same number of C4b,2a sites (Fig. 4). A similar experiment was performed to examine the effects of CR1 on $EAC14^{oxy}235_{lim}$ (0.7 sites/cell). In contrast to its inhibitory activities on $EAC142_{lim}$ and $EAC1423_{lim}$, CR1 was totally inactive on $EAC14235_{lim}$ even at concentrations of 800 ng/ml (data not shown).

Activity of CR1 on C4b in the Fluid Phase. The previous experiments demonstrated that, like C4-bp, CR1 promotes the decay-dissociation of the classical pathway C3 convertase. Fig. 8 shows, in addition, that CR1 and C4-bp have analogous activities on fluid phase C4b, because both function as cofactors for the cleavage of C4b. In the presence of CR1 (or C4-bp), C3b/C4b inactivator cleaves the α′ chain of C4b into

three fragments, $\alpha 2$, $\alpha 3$, and $\alpha 4$, with apparent molecular weights of 44,000, 27,000, and 17,000, respectively. By SDS-PAGE, the fragments originating from the cofactor activities of CR1 and C4-bp are indistinguishable. From the results of several experiments, the cofactor activity of C4-bp is between two and four times higher than that of CR1 on a weight basis.

Discussion

It has been shown previously that CR1 inhibits the alternative pathway C3 convertase (C3b,Bb,P) by enhancing the decay of Bb (9). The main finding of this paper is that CR1 also inhibits the C3 and C5 convertases of the classical pathway (C4b,2a and C4b,2a,3b).

In purifying functional CR1 to homogeneity, we monitored activity with two assays, i.e., the inhibition of hemolytic function of C4b,2a and C3b,Bb,P assembled on the erythrocyte surface. We found throughout the isolation procedure that both activities were similarly distributed. The final preparations showed a single polypeptide chain in either the reduced or unreduced form, with a molecular weight of ~200,000 (Fig. 1). At low concentrations, the purified protein inhibited the consumption of C3 by C4b,2a (Fig. 2). On the basis of these findings, we conclude that CR1 inhibits the classical pathway C3 convertase, an enzyme whose active site is in C2a, but whose function is C4b dependent.

Other cell-associated decay-accelerating factors of the classical pathway C3 convertase have previously been described. Several years ago, Hoffman found such an activity in crude butanol extracts of stroma from human red cells (29). More recently, Burge et al (30) isolated an inhibitor of C4b,2a from guinea pig erythrocytes. Its properties are similar to those of CR1, which suggests that they may be homologous proteins.

The mechanism of inhibition of C4b,2a by CR1 probably consists of competitive displacement of C2a from C4b. This is indicated by observations that the purified receptor enhances the decay of the hemolytic activity of EAC142 (Fig. 3) and that ^{oxy}C2 inhibits the binding of ^{125}I-CR1 to EAC14 cells (Fig. 5). The possibility of direct effects of CR1 on C4b was excluded in experiments showing that CR1 alone did not inhibit the hemolytic activity of $EAC14_{lim}$, nor did it enhance the inactivation of the cell-bound C4b by C3b/C4b inactivator (Fig. 6).

It is of interest that the overall effects of CR1 and the serum protein C4-bp on EAC14, EAC142, and fluid phase C4b are similar, although quantitative differences were noticed. Neither protein irreversibly influences the hemolytic function of $EAC14_{lim}$; both promote decay of EA142, and both serve as cofactors for the cleavage of fluid phase C4b. However, on a weight basis, CR1 is a much better inhibitor of EAC142 than C4-bp (Fig. 4), although as a cofactor, C4-bp is apparently more efficient (Fig. 8).

CR1 is also a powerful inhibitor of the classical pathway C5 convertase $EAC14^{oxy}23_{lim}$. In this complex enzyme, the proteolytic site resides on the C2a subunit and C3b provides an essential anchorage site for the substrate C5 (31–34). Although we have not further investigated the mechanisms whereby CR1 inhibits C5 convertase, it is likely that CR1 competes with the substrate C5 for a binding site on the C3b subunit of the enzyme. Under the experimental conditions used, an effect of CR1 on the other subunits, $C4b^{oxy}2a$, seems less likely, because these components were present

in great excess on the erythrocytes. Moreover, the inhibitory effects on $EAC14^{oxy}23_{lim}$ were detected at concentrations of CR1 below those necessary to inactivate $EAC14^{oxy}2_{lim}$.

In short, CR1 controls the activity of all amplifying enzymes of the complement cascade that are C3b or C4b dependent, presumably by decay-dissociation of C2 (or factor B), by inhibiting the C3b-C5b interaction, or by serving as a cofactor for the cleavage of the α' chain of C4b (and C3b) by C3b/C4b inactivator. Therefore, CR1 is a powerful membrane-associated inhibitor of the complement system whose functional properties encompass those of the plasma proteins C4-bp and β1H (Table I).

Because CR1 inhibits C3b,Bb,P, Fearon (9) proposed that the presence of CR1 on cell membranes may prevent inappropriate self recognition by complement. This hypothesis was put forward in the context of recent insights into the mechanisms of activation of the alternative pathway (43–45). According to these ideas, low level deposition of C3b, through its metastable binding site, occurs continuously in vivo, both on autologous cells and on foreign particles. Whether the cascade will proceed or not depends on the interaction between C3b and certain cell surface structures not yet well characterized. One of these structures, according to Fearon, might be CR1, which would bind to C3b already deposited on another site of the membrane and prevent the formation of an intrinsic C3 convertase, C3b,Bb. This hypothesis necessitates both fruitful and rapid collisions between the two membrane-associated moieties.

On the basis of the present findings, it is our view that CR1 can function as an inhibitor of enzymes extrinsic to the cells bearing the receptor. A well-known function of CR1 is to promote adherence of C3b- and C4b-bearing substrates to phagocytes. This interaction, however, could result in a microenvironment potentially damaging to the plasma membrane of the responding cell. Thus, complement-bearing immune complexes or bacteria, in close proximity to the host cell, might promote local activation and deposition of complement components, with attendant lethal consequences for the host cell. Seen in this context, the ability of some phagocytic cells to synthesize and secrete several complement components, including C3 and C4, might aggravate these effects.

We therefore wish to propose that CR1 on the surface of cells supplies an increased local concentration of a strong inhibitor of complement activation, and, therefore, provides cells with a mechanism for circumventing damage, while at the same time

TABLE I

Comparison of Properties of CR1, C4-bp, and β1H

Properties		CR1*	C4-bp‡	β1H§
Inhibition of hemolytic activity of particle associated	C4b,2a	Yes	Yes	No
	C4b,2a,3b	Yes	ND‖	Yes
	C3b,Bb	Yes	No	Yes
Cofactor activity for the cleavage of fluid phase	C4b	Yes	Yes	No
	C3b	Yes	Yes	Yes

* Data in this paper and in reference 9.
‡ Data in references 27, 35–38.
§ Data in references 35, 39–42.
‖ Not determined.

interacting with complement-bearing substrates. The view that complement receptors are also complement inhibitors suggests new ideas about their role on the membrane of nonphagocytic cells. For example, it raises the possibility that CR1 protects B lymphocytes from the damaging effects of the complement activation that might occur in their vicinity, after the specific binding of antigen or of antigen-antibody complexes to Fc receptors.

In summary, the present observations show that complement receptors of cell membranes are functionally homologous to the plasma proteins β1H and C4-bp, and suggest that these receptors or similar structures may provide a selective advantage to cells or organisms that, at some time during their life history, may come in close contact with antigen-antibody complexes and complement. Of course, further experiments are necessary to test this hypothesis directly.

While this paper was being prepared for submission, findings similar to ours were brought to our attention (46).

Summary

A glycoprotein from the membrane of human erythrocytes has been identified as a receptor for C3b (CR1). It promotes the dissociation of the alternative pathway C3 convertase C3b,Bb and the cleavage of C3b by C3b/C4b inactivator. We find that CR1 also inactivates the C3 and C5 convertases of the classical pathway.

CR1 inhibits the consumption of C3 by C3 convertase EAC142 and enhances the decay of C4b,2a sites. On a weight basis, CR1 is ~5–10 times more active than C4 binding protein, a serum inhibitor of C4b,2a. The binding of ^{125}I-CR1 to EAC14 cells is inhibited by C2. Therefore, it is likely that CR1 and C2 compete for a site on C4b. CR1 inhibited C5 convertase even more effectively, but had no effect on the assembly of the late complement components.

At high concentrations, CR1 alone has no irreversible effects on cell-bound C4b. In the fluid phase, CR1 can function as a cofactor for the cleavage of the α' chain of C4b by C3b/C4b inactivator.

A well-known function of CR1 is to promote adherence of microbes or immune complexes bearing C3b and C4b to cells. This interaction could result in a microenvironment damaging to the plasma membrane of the responding cell because the extrinsic C3b and C4b fragments can serve as additional sites of assembly of enzymes of the cascade. We therefore wish to propose that CR1 on the surface of cells supplies an increased local concentration of a strong inhibitor of the amplifying enzymes of the complement system and provides cells with a mechanism for circumventing damage when they bind C3b- and C4b-bearing substrates.

The authors thank Richard Melton for excellent technical participation; Joanne Joseph for manuscript preparation; and Drs. M. Lamm, J. Phillips-Quagliata, and L. Hall for their editorial suggestions.

Received for publication 12 December 1980.

References

1. Nelson, R. A. 1953. The immune-adherence phenomenon. An immunologically specific reaction between microorganisms and erythrocytes leading to enhanced phagocytosis. *Science (Wash. D. C.).* **118**:733.

2. Bianco, C., R. Patrick, and V. Nussenzweig. 1970. A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. *J. Exp. Med.* **132:**702.
3. Lay, W. H., and V. Nussenzweig. 1968. Receptors for complement on leukocytes. *J. Exp. Med.* **128:**991.
4. Huber, H., M. J. Polley, W. D. Linscott, H. H. Fudenberg, and H. J. Müller-Eberhard. 1968. Human monocytes: distinct receptors for the third component of complement and for immunoglobulin G. *Science (Wash. D. C.).* **162:**1281.
5. Gigli, I., and R. A. Nelson, Jr. 1968. Complement dependent immune phagocytosis. I. Requirements for C'1, C'4, C'2 and C'3. *Exp. Cell. Res.* **51:**45.
6. Gelfand, M. C., M. M. Frank, and I. Green. 1975. A receptor for the third component of complement in the human renal glomerulus. *J. Exp. Med.* **142:**1029.
7. Mantovani, B., M. Rabinovitch, and V. Nussenzweig. 1972. Phagocytosis of immune complexes by macrophages: different roles of the macrophage receptor sites for complement (C3) and for immunoglobulin IgG. *J. Exp. Med.* **135:**780.
8. Ehlenberger, A. G., and V. Nussenzweig. 1977. The role of membrane receptors for C3b and C3d in phagocytosis. *J. Exp. Med.* **145:**357.
9. 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. U. S. A.* **76:**5867.
10. Fearon, D. T. 1980. Identification of the membrane glycoprotein that is the C3b receptor of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte and monocyte. *J. Exp. Med.* **152:**20.
11. Cooper, N. R. 1969. Immune adherence by the fourth component of complement. *Science (Wash. D. C.).* **165:**396.
12. Ross, G. D., and M. J. Polley. 1975. Specificity of human lymphocyte complement receptors. *J. Exp. Med.* **141:**1163.
13. Hoffmann, L. G., and M. M. Mayer. 1977. Immune hemolysis and complement fixation. *Methods Immunol. Immunochem.* **4:**137.
14. Sakai, K., and R. M. Stroud. 1973. Purification, molecular properties, and activation of C1-proesterase, C1s. *J. Immunol.* **110:**1010.
15. Gigli, I., I. von Zabern, and R. R. Porter. 1977. The isolation and structure of the fourth component of human complement. *Biochem. J.* **165:**439.
16. Tack, B. F., and J. W. Prahl. 1976. Third component of human complement: purification from plasma and physicochemical characterization. *Biochemistry.* **15:**4513.
17. Scharfstein, J., A. Ferreira, I. Gigli, and V. Nussenzweig. 1978. Human C4-binding protein. I. Isolation and characterization. *J. Exp. Med.* **148:**207.
18. Fearon, D. T. 1977. Purification of C3b inactivator and demonstration of its two polypeptide chain structure. *J. Immunol.* **119:**1248.
19. Götze, O., and H. J. Müller-Eberhard. 1971. The C3 activator system: an alternative pathway of complement activation. *J. Exp. Med.* **134:**90s.
20. Penskey, J., C. F. Hinz, Jr., E. W. Todd, R. J. Wedgwood, J. T. Boyer, and I. H. Lepow. 1968. Properties of highly purified human properdin. *J. Immunol.* **100:**142.
21. Nagasawa, S., and R. M. Stroud. 1977. Cleavage of C2 by C1s into the antigenically distinct fragments C2a and C2b: demonstration of binding of C2b to C4b. *Proc. Natl. Acad. Sci. U. S. A.* **74:**2998.
22. Polley, M. J., and H. J. Müller-Eberhard. 1967. Enhancement of the hemolytic activity of the second component of complement by oxidization. *J. Exp. Med.* **126:**1013.
23. Nelson, R. A., J. Jensen, I. Gigli, and N. Tamura. 1966. Methods for separation, purification and measurement of nine components of hemolytic complement in guinea pig serum. *Immunochemistry.* **3:**111.

24. Cooper, N. R., and H. J. Müller-Eberhard. 1970. The reaction mechanism of C5 in immune hemolysis. *J. Exp. Med.* **132**:775.
25. Maddy, A. H., and R. L. Spooner. 1970. Ox erythrocyte agglutinability. I. Variation in the membrane protein. *Vox Sang.* **18**:34.
26. Laemmli, J. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* **227**:680.
27. 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. U. S. A.* **76**:6596.
28. Pangburn, M. K., R. D. Schreiber, and H. J. Müller-Eberhard. 1978. Molecular interactions of the control proteins C3b inactivator and β1H with various complement intermediates. *J. Immunol.* **120**:1791. Abstr.
29. Hoffmann, E. M. 1969. Inhibition of complement by a substance isolated from human erythrocytes. I. Extraction from human erythrocyte stromata. *Immunochemistry.* **6**:391.
30. Burge, A. N., M. Rowe, and K. F. Austen. 1980. Isolation from guinea pig serum and guinea pig erythrocyte stromata of two distinct decay accelerating factors for the classical pathway C3 convertase. *J. Immunol.* **124**:1516. Abstr.
31. Goldlust, M. B., H. S. Shin, C. H. Hammer, and M. M. Mayer. 1974. Studies of complement complex C5b,6 eluted from EAC-6: reaction of C5b,6 with EAC4b,3b and evidence on the role of C2a and C3b in the activation of C5. *J. Immunol.* **113**:998.
32. Shin, H. S., R. J. Pickering, and M. M. Mayer. 1971. The fifth component of the guinea pig complement system. II. Mechanism of SAC1,4,2,3,5b formation and C5 consumption by EAC1,4,2,3. *J. Immunol.* **106**:473.
33. Shin, H. S., R. J. Pickering, and M. M. Mayer. 1971. The fifth component of the guinea pig complement system. III. Dissociation and transfer of C5b, and the probable site of C5b fixation. *J. Immunol.* **106**:480.
34. Cooper, N. R., and H. J. Müller-Eberhard. 1970. Reaction mechanism of human C5 in immune hemolysis. *J. Immunol.* **132**:775.
35. 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.
36. Fujita, T., I. Gigli, and V. Nussenzweig. Human C4-binding protein. II. Role in proteolysis of C4b by C3b inactivator. *J. Exp. Med.* **148**:1044.
37. Shiraishi, S., and R. M. Stroud. 1975. Cleavage products of C4b produced by enzymes in human serum. *Immunochemistry.* **12**:935.
38. Nagasawa, S., and R. M. Stroud. 1977. Mechanism of action of the C3b inactivator: requirement for a high molecular weight cofactor (C3b-C4bINA cofactor) and production of a new C3b derivative (C3b'). *Immunochemistry.* **14**:749.
39. Nagaki, K., K. Iida, M. Okubo, and S. Inai. 1978. Reaction mechanisms of β1H globulin. *Int. Arch. Allergy Appl. Immunol.* **57**:221.
40. Whaley, K., and S. Ruddy. 1976. Modulation of C3b hemolytic activity by a plasma protein distinct from C3b inactivator. *Science (Wash. D. C.).* **193**:1011.
41. Weiler, J. M., M. R. Daha, K. F. Austen, and D. T. Fearon. 1976. Control of the amplification convertase of complement by the plasma protein β1H. *Proc. Natl. Acad. Sci. U. S. A.* **73**:3268.
42. Pangburn, M. K., R. D. Schreiber, and H. J. Müller-Eberhard. 1977. Human complement C3b inactivator: isolation, characterization, and demonstration of an absolute requirement for the serum protein β1H for cleavage of C3b and C4b in solution. *J. Exp. Med.* **146**:257.
43. Fearon, D. T., and K. F. Austen. 1977. Activation of the alternative complement pathway due to resistance of zymosan bound amplification convertase to endogenous regulatory mechanisms. *Proc. Natl. Acad. Sci. U. S. A.* **74**:1683.
44. Pangburn, M. K., and H. J. Müller-Eberhard. 1978. Complement C3 convertase: cell

surface restriction of β1H control and generation of restriction on neuraminidase-treated cells. *Proc. Natl. Acad. Sci. U. S. A.* **75:**2416.

45. Nicol, P. A. E., and P. J. Lachmann. 1973. The alternate pathway of complement activation. The role of C3 and its inactivator (KAF). *Immunology.* **24:**259.
46. Gigli, I., and D. T. Fearon. 1981. Regulation of the classical pathway convertase of human complement by the C36 receptor of human erythrocytes. *Fed. Proc.* **40:**1172 (abstr.).