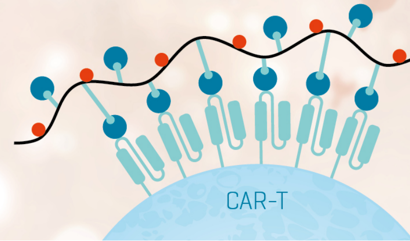
CAR-T Cell Quantification with Dextramer® Technology
Choose Your Target Antigen
We Make the Reagent for You
LEARN MORE



The Journal of Immunology

RESEARCH ARTICLE | JANUARY 15 1991

Proposed structure of the F' allotype of human CR1. Loss of a C3b binding site may be associated with altered function. FREE

W W Wong; ... et. al

J Immunol (1991) 146 (2): 656–662.
https://doi.org/10.4049/jimmunol.146.2.656

Related Content

Natural antibody and complement-mediated antigen processing and presentation by B lymphocytes.
J Immunol (February,1994)

Proteins involved in prophenoloxidase activating system and their roles in mud crab Scylla serrata (53.12)
J Immunol (April,2007)

Somatic Hypermutation of an Artificial Test Substrate Within an Igκ Transgene
J Immunol (July,1998)

Downloaded from http://journals.aai.org/jimmunol/article-pdf/146/2/656/1050117/656.pdf by guest on 26 April 2025

0022-1767/91/1462-0656$02.00/0
THE JOURNAL OF IMMUNOLOGY
Copyright © 1991 by The American Association of Immunologists
Vol. 146, 656-662, No. 2, January 15, 1991
Printed in U.S.A.

PROPOSED STRUCTURE OF THE F′ ALLOTYPE OF HUMAN CR1

Loss of a C3b Binding Site May Be Associated with Altered Function[1]

WINNIE W. WONG[2*†] AND SCOTT A. FARRELL*

*From the *Department of Rheumatology and Immunology, Brigham and Women's Hospital, and the †Department of Medicine, Harvard Medical School, Boston, MA 02115*

Human CR1 is composed of tandem long homologous repeating (LHR) segments that encode separate binding sites for C3b or C4b. Homologous recombination with unequal crossover has been proposed as the genetic mechanism that gave rise to the CR1 alleles that differed in their total numbers of LHR. The F allotype has four LHR, named LHR-A, -B, -C, -D, 5′ to 3′. The site in LHR-A preferentially binds C4b and those in LHR-B and -C prefer C3b. A previous study revealed the presence of a fifth LHR with sequences similar to LHR-B and a third C3b binding site in the S allotype of higher m.w. In the present study, an 18-kb *Eco*RV fragment that was associated with the expression of the lower m.w. F′ allotype hybridized with a unique pattern of cDNA and intron probes specific for LHR-C. Deletion of LHR-B and one C3b binding site was proposed as the mechanism for the appearance of this F′-specific fragment. Functional differences among the CR1 variants were sought by comparative analyses of soluble rCR1 having one, two or three C3b binding sites. Although these three variants did not exhibit any significant differences in their capacities to act as cofactors for the cleavage of monomeric C3b, their relative affinities for dimeric ligand varied more than 100-fold. Furthermore, the variant with only one C3b binding site was at least 10-fold less effective in the inhibition of the alternative pathway C3 and C5 convertases. These observations suggested that the F′ allotype may be impaired in its capacity to bind opsonized immune complexes, to inhibit the formation of the alternative pathway C3 and C5 convertases, and perhaps to mediate other CR1-dependent cellular responses.

The wide distribution of human CR1 (CD35) on almost all peripheral blood cells suggests that it plays multiple roles in host defense. In addition to the adherence and removal of C3- and C4-coated immune complexes and microorganisms, this receptor has been implicated in the modulation of the immune response by the observations that cross-linking of CR1 leads to activation of B lymphocytes and secretion of IL-1 by monocytes (1–3). Its high affinity for the C3b and C4b ligands underlies its capacity to displace the Bb and C2a fragments from the alternative and classical pathway C3 and C5 convertases (4, 5). The therapeutic potential of this molecule as an inhibitor of complement-mediated inflammatory reactions is supported by a recent study in which soluble rCR1 reduced the sizes of myocardial infarcts in a rat model of reperfusion injury (6).

The primary structure of human CR1 has been deduced from sequence analysis of the cDNA and its extracytoplasmic domain is composed entirely of the sixty to seventy amino acid repeats (SCR[3]) that are typical of the complement regulatory proteins and receptors on chromosome 1 (7). In CR1, groups of seven of these smaller repetitive units are tandemly organized into LHR that encode separate binding sites for C3b or C4b (8, 9). Four allotypic forms of CR1 that differ in size by 30- to 50-kDa increments have been described. The transcripts that are associated with each allotype also differ in increments of ~1.4 kb, indicating that their primary sequences vary in the number of LHR (10–15). There are four LHR in the F (or A) allotype of ~250 kDa, termed LHR-A, -B, -C, and -D, respectively, 5′ to 3′ (8, 9, 16). Although the first two SCR in LHR-A determine its ability to bind C4b, the corresponding units in LHR-B and -C determine their higher affinities for C3b (8, 9). Analysis of the gene encoding the larger S (or B) allotype of ~290 kDa by restriction mapping of genomic phage clones revealed a fifth LHR that is a chimera of the 5′ half of LHR-B and the 3′ half of LHR-A and is predicted to contain a third C3b binding site (16). The smallest F′ (or C) allotype of CR1 of ~210 kD, found in increased incidence in patients with SLE and is associated with patients in multiple lupus families (12, 17) may have resulted from the deletion of one LHR and may be impaired in its capacity to bind efficiently to immune complexes coated with complement fragments. Functional analyses of the CR1 variants were initially performed by Seya et al. (18) in which purified S and F forms and partially purified F′ forms were compared in their cofactor activities and in the inhibition of fluid phase classical and alternative pathway C3 convertases. Although no significant differences were observed, these earlier studies were not designed to detect any differences in the capacities of the CR1 variants to bind dimeric ligands.

The present study defines the molecular basis of the F′

Received for publication July 6, 1990.
Accepted for publication October 18, 1990.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by Grants AR-37408 and AI-23401 from the National Institute of Health.
[2] Address correspondence and reprint requests to Dr. W. Wong, BASF Bioresearch Corporation, 195 Albany Street, Cambridge, MA 02139.

[3] Abbreviations used in this paper: SCR, short consensus repeat; LHR, long homologous repeat.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/146/2/656/1050117/656.pdf by guest on 26 April 2025

allele by using intron probes specific for each LHR to analyze an *Eco*RV RFLP of the CR1 that is present in individuals who express this allotype. Furthermore, soluble rCR1 variants having one, two, or three C3b recognition sites and corresponding to the predicted structures of the F′, F, and S allotypes, respectively, were compared for their abilities to bind dimeric ligand, to act as cofactor for the cleavage of C3b, and to inhibit the substrate-bound alternative and classical pathway C3 and C5 convertases.

MATERIALS AND METHODS

Analysis of DNA. Genomic DNA was prepared from peripheral blood leukocytes, digested with *Eco*RV, electrophoresed and analyzed by Southern blotting as previously described (14). The CR1 cDNA probe 1-1 hybridizes to SCR-4 to -7 of all the LHR whereas the probe 1-4 specifically hybridizes to SCR-1 and -2 of LHR-B and -C (8, 9, 16). The noncoding probe PE hybridizes to the intron between the two exons that encode SCR-2 in LHR-B and -C. PX hybridizes to the intron between the two exons that encode SCR-6 in LHR-A and -B and HE hybridizes to the intron 5′ of SCR-7 in LHR-A and -B (16) (Fig. 2). A genomic library derived from the DNA of an individual homozygous for the F allele was screened for CR1 clones by hybridization to cDNA probes that spanned the entire coding sequence. Restriction mapping of the overlapping phage clones that spanned the F allele was performed as previously described (16).

Construction of expression plasmids and purification of soluble rCR1. A Pst I fragment which extended from SCR-5 in LHR-A to a corresponding position in LHR-B was isolated from the plasmid pBSABCD that contained the complete coding sequence of the F allotype of CR1 (9). This was inserted into piABCD (9) which had been linearized by partial digestion with *Pst*I, resulting in the creation of a fifth LHR with coding sequences identical to that of LHR-B. A clone named piABBCD with an in-frame insertion was selected by restriction mapping and the segment encoding the entire extracellular domain was excised by digestion with *Xho*I and *ApaL* I and treated with the Klenow DNA polymerase. The blunt-ended fragment was ligated to the linkers, 5′-TGAGCTAGCTCA-3′, digested with *Nhe*I, and inserted into the *Xba*I site of the expression vector Ap^{r}M8, a derivative of CDM8 (19). This plasmid, named pasecABBCD, had a stop codon inserted after the 37th SCR and lacked the sequences encoding the transmembrane and cytoplasmic domains. The plasmid, pasecABCD, was made by the transfer of the four LHR of the F allotype from pBSABCD into Ap^{r}M8 using a similar strategy. The plasmids piABCD, pasecABCD, and Ap^{r}M8 were gifts of Dr. Lloyd Klickstein (Center for Blood Research, Boston, MA). A third plasmid, containing only three LHR and named pasecACD, was made by an in-frame deletion of the *Pst*I fragment extending from SCR-5 in LHR-A to a corresponding position in LHR-B.

A total of 30 to 60 μg of each of the above plasmids was used to transfect 2 × 10^7 COS-1 cells (American Type Culture Collection, Rockville, MD) in the presence of 400 μg/ml DEAE-dextran, and 100 μM chloroquine for 4 h at 37°C in DMEM with high glucose (Hazelton, Lenexa, KS) and 10% Nuserum (Collaborative Research, Bedford, MA). The cells were shocked for 3 min at room temperature with 10% DMSO in HBSS without divalent cations after removal of the transfection medium (20), washed and cultured in DMEM and 10% FCS. The culture supernatants were collected every 48 h for 10 days, clarified of cell debris by centrifugation, and frozen at −70°C. On thawing of the supernatants, PMSF and sodium azide were added to final concentrations of 5 mM and 0.2%, respectively, and rCR1 was purified by affinity chromatography on mAb YZ-1-Sepharose as described except that detergents were omitted from the eluting buffer (21). The purified proteins were dialyzed twice against 1000 volumes of PBS and frozen in small aliquots at −70°C. This procedure routinely yielded 150 to 200 μg of rCR1 as determined by the Micro BCA kit (Pierce, Rockford, IL) using BSA as a standard. The protein was analyzed by SDS-PAGE on a gel containing a linear gradient of 5% to 15% acrylamide.

Cofactor activity of rCR1. Purified human C3 (22) was treated with 0.5% TPCK-trypsin (Sigma Chemical Co., St. Louis, MO) for 5 min at 37°C and the reaction was stopped by the addition of a fourfold molar excess of soy bean trypsin inhibitor. The C3b was labeled with ^{125}I to a sp. act. of 5 × 10^5 cpm/μg using Iodogen (Pierce Chemical Co., Rockford, IL). Cofactor activity of rCR1 was assessed by incubation of 200 ng of C3b, 100 ng of factor I (23) with varying amounts of rCR1 in 20 μl PBS for 1 h at 37°C (21). The reactions were stopped by boiling the samples in an equal volume of SDS-PAGE sample buffer containing 0.1 M dithiothreitol. After electrophoresis and autoradiography, the areas of the dried gel corresponding to the positions of the α′ chains were excised and the amount of radioactivity measured in a Beckman gamma-counter (Beckman Instrument, Inc., Fullerton, CA) (21). The counts associated with the α′ chain in the absence of CR1 was taken as the 100% control.

Capacity of rCR1 to bind dimeric C3b. C3b was cross-linked by dimethyl suberimidate (Sigma) (24) or 1,6-bismaleimidohexane (Pierce) (6) and dimers were selected by sedimentation on a linear gradient of 7.5 to 30% sucrose in PBS (24). Either method yielded dimers that bound to erythrocyte CR1 with association constants (K_a) that ranged from 1 to 3 × 10^8 M^{-1}. A total of 300 ng of ^{125}I-C3b dimers (4 × 10^6 cpm/mg) were incubated with 2 × 10^8 E in 200 μl HBSS with 0.1% BSA in the absence or presence or incremental amounts of unlabeled monomeric or dimeric C3b, or the different soluble forms of rCR1 (6). After 1 h on ice, cell-bound ligand was separated from unbound material by centrifugation of the E through dibutylphthalate (24). The amount of dimeric C3b bound in the presence of excess rabbit IgG anti-CR1 was taken as the nonspecific background and specific counts bound in the absence of any inhibitor was used as the 100% control. For all these binding studies, erythrocytes from one normal individual were utilized. These cells were homozygous for the F allotype and had relatively high amounts of CR1 (~800 YZ-1 mAb binding sites).

Inhibition of alternative and classical pathway convertases. For assessment of the activation of the alternative pathway, 25% human serum was incubated with 5 × 10^6 zymosan particles (gift of Dr. Joyce Czop, Harvard Medical School, Boston, MA) in Veronal-buffered saline with 2 mM $MgCl_2$ and 8 mM EGTA in the absence or presence of incremental amounts of rCR1. For assessment of the activation of the classical pathway, 60 μg/ml of heat aggregated rabbit IgG was substituted for zymosan and the reactions were performed in Veronal-buffered saline with 0.5 mM $MgCl_2$ and 0.15 mM $CaCl_2$ (6). After incubation for 40 min at 37°C, the reactions were stopped by addition of 10 mM EDTA and assayed for the amounts of C3a and C5a cleavage using RIA kits (Amersham, Chicago, IL).

RESULTS

Structure of the F′ allele of CR1. We have previously reported that when the DNA of individuals who expressed the ~210 kDa F′ allotype of CR1 was digested with *Eco*RV, an additional fragment of 18 kb was observed on probing the Southern blots with the CR1 cDNA probe 1-1 (14) (Fig. 1). This probe was originally derived from SCR-3 to SCR-7 in LHR-B but its sequences were sufficiently homologous to allow hybridization to the fourth through the seventh SCR of other LHR (8, 9, 16). To assign each fragment to an LHR, the overlapping genomic clones that spanned the entire F allele were mapped by *Eco*RV (Fig. 2). Fragments of 9.4 and 22 kb corresponded to those expected from LHR-A and -D, respectively. This

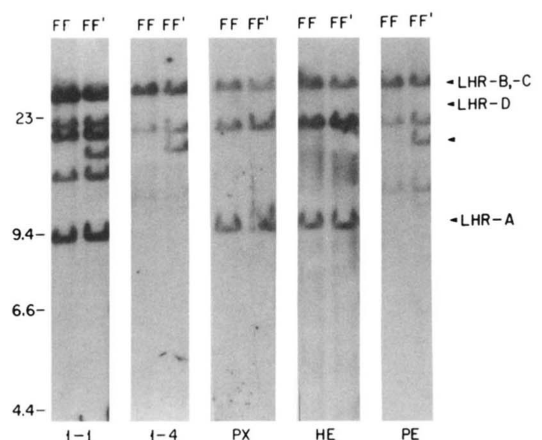


Figure 1. Autoradiographs of the Southern blots in which the CR1 cDNA and intron probes were hybridized to the *Eco*RV digests of the DNA from individuals who expressed the F or the F′ allotypes. The positions of the *Hin*dIII fragments of lDNA are designated in kb on the left. The position of the F′-specific fragment is designated by a *single arrow*.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/146/2/656/1050117/656.pdf by guest on 26 April 2025

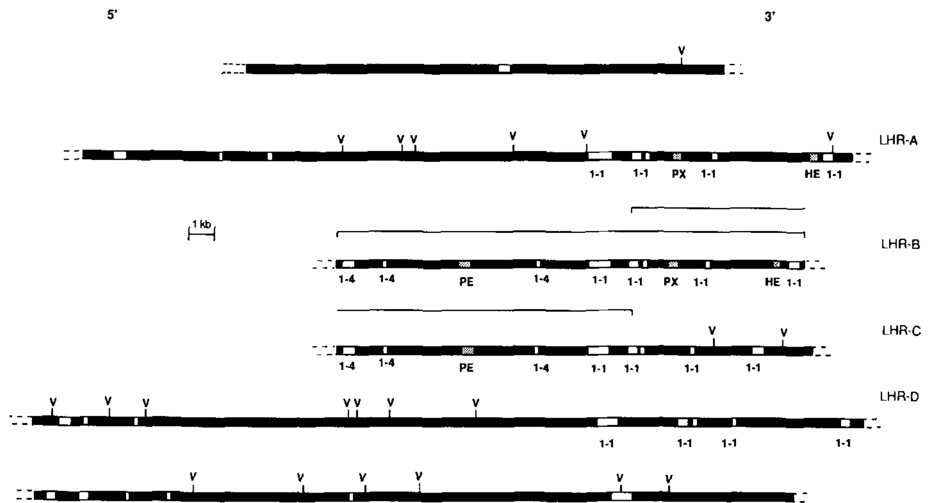


Figure 2. *Eco*RV restriction map of the F allele of CR1. The *white boxes* represent the positions of the exons and the *stippled boxes* represent the sites of hybridizations of the intron probes. The *brackets* over LHR-B and -C indicate two possible regions of deletion. V represents an *Eco*RV site.

was confirmed by the hybridization of the 9.4-kb but not the 22-kb fragment to the intron probes PX and HE (Fig. 1). Inasmuch as there were no *Eco*RV sites in LHR-B, the largest fragment at the top of the blot that hybridized to all the probes represented the ~32-kb fragment that spanned LHR-B and most of LHR-C (Figs. 1 and 2). Restriction mapping of genomic clones that spanned the previously reported CR1-like pseudogene (data not shown) (16) indicated that the fragments of 20 and 15 kb were derived from this region (Fig. 1).

The 18-kb *Eco*RV fragment that was associated with the expression of the F′ allotype hybridized to the cDNA probe 1-4 and the intron probe PE, indicating that it contained the 5′ half of LHR-B or -C. This fragment did not hybridize to the intron probes PX and HE indicating that it lacked the 3′ half of LHR-B (Fig. 1). Deletion of LHR-B or another fragment of similar length from the ~32 kb *Eco*RV fragment would yield a fragment of 18 kb that extended from the 3′ most *Eco*RV site of LHR-A to the 5′ most *Eco*RV site of LHR-C (Fig. 2). Such a fragment would be expected to hybridize only with the probes 1-4, PE, and 1-1, consistent with the findings in Figure 1. Inasmuch as any ~15-kb deletion including the PX and HE sites of LHR-B necessarily includes at least one of the exons that contain the C3b binding site in either LHR-B or -C, the resulting allele of three LHR would encode only one C3b binding site.

Plasmid construction and purification of soluble rCR1. Previous measurements of the uptake of dimeric C3b by cell bound-CR1 did not reveal any significant differences in the affinities of the F and S forms (10) because the cross-linking of adjacent CR1 molecules could not be discerned from the binding via two sites within one molecule of CR1. Recent findings by Weisman et al. (6) demonstrated that soluble CR1 could bind C3b dimers via tandem binding sites and provided a direct way to evaluate the respective affinities of the CR1 variants for dimeric ligand. cDNA constructs for soluble CR1 were made by the insertion of a stop codon between the end of the 3′ most SCR and the beginning of the transmembrane domain. Based on the results of the genomic analyses, we produced rCR1 with structures predicted for the different polymorphic variants. Our strategy for the insertion or deletion of an LHR used the conserved restriction sites to preserve the reading frame. The *Pst*I fragment that was inserted or deleted encoded SCR-5, -6, and -7 of LHR-A and SCR-1, -2, -3, and -4 of LHR-B (Fig. 3). Inasmuch as the amino acid sequences of the third to the seventh SCR are identical in LHR-A and -B (Fig. 3) (8, 9), these procedures would result in the insertion or deletion of sequences equivalent to an LHR-B. The plasmids pasecACD, pasecABCD, and pasecABBCD (Fig. 3) would therefore encode proteins that have one, two or three C3b binding sites, respectively.

Soluble rCR1 was isolated by chromatography on YZ-1-Sepharose from the culture supernatants of COS cells that had been transfected with the CR1 plasmids. Each rCR1 protein was more than 95% pure and the three forms exhibited incremental M_r differences of ~30 kDa under nonreducing conditions on SDS-PAGE, similar to those observed for the naturally occurring allotypes (Fig. 4). Biosynthetic labeling with ^{35}S-cysteine of the COS cells transfected with the vector Ap^{r}M8 alone showed no adsorption of CR1-like proteins to the YZ-1-Sepharose (data not shown). Furthermore, the soluble rCR1 had M_r that were similar to the CR1 isolated from ^{125}I-labeled erythrocytes (data not shown), consistent with the deletion of only 70 amino acids from each molecule. In the lanes that contained rCR1 isolated from pasecABBCD- or pasecABCD-transfected cells, small amounts of protein with M_r similar to the smaller forms were observed (Fig. 4, *lanes 2* and *3*). These may represent the products of homologous recombination that were spontaneously generated within the transfected COS cells.

Cofactor activity of rCR1 The functional integrity of the different forms of rCR1 was measured in a cofactor assay in which radiolabeled C3b was converted to the iC3b and C3dg fragments. The amounts of rCR1 that were required for 50% factor-I-mediated cleavage of the α' chain of C3b ranged from 3.5 nM for the pasecABBCD-derived protein to 8 nM for the pasecACD-derived protein and differed only slightly for the three forms (Fig. 5). Furthermore, conversion of C3b to C3dg was seen with the addition of 10 to 20 nM of all forms of rCR1 (data not shown). Thus soluble rCR1, irrespective of the number of C3b binding sites, retained the capacity of the native molecule to bind C3b and served as a cofactor for the factor-I mediated cleavage.

Capacity of rCR1 to bind dimeric C3b. To assess the effect of having different numbers of LHR-B on the affin-

Downloaded from http://journals.aai.org/jimmunol/article-pdf/146/2/656/1050117/656.pdf by guest on 26 April 2025

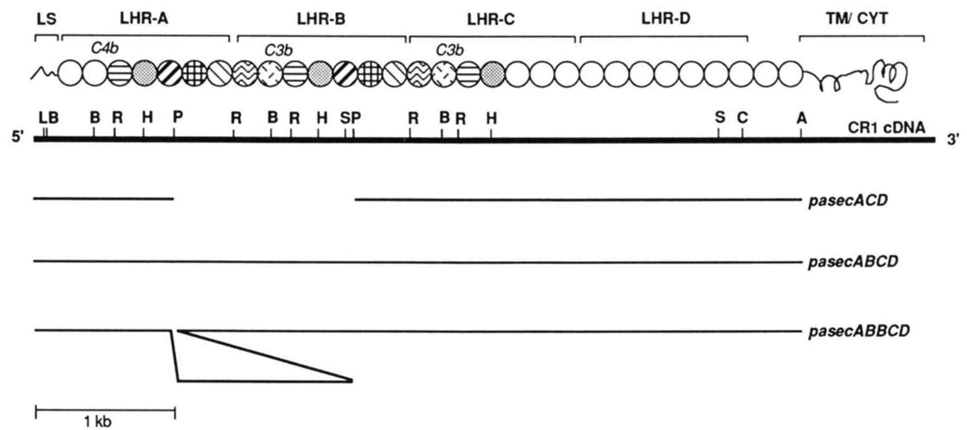


Figure 3. The cDNA inserts for the different forms of soluble rCR1. The restriction sites shown are: A, *ApaLI*; B, *BamHI*; C, *SacI*; H, *HindIII*; L, *BglI*; P, *PstI*; R, *EcoRI*; and S, *SmaI*. The cartoon at the top represents the CR1 peptide, and the SCR with identical sequences are filled in by the same patterns.

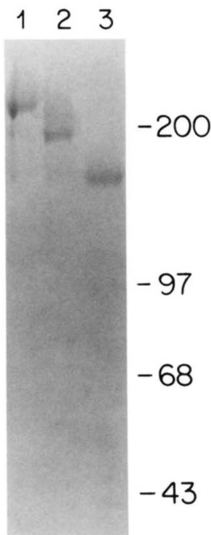


Figure 4. Coomassie blue-stained SDS-PAGE under nonreducing conditions of soluble rCR1 purified by adsorption on YZ-1-Sepharose. Each lane contains 10 μg of rCR1 purified from the culture supernatants of COS cells that have been transfected with pasecABBCD (*lane 1*), pasecABCD (*lane 2*), or pasecACD (*lane 3*). The positions of the M_r markers are indicated on the right in kDa.

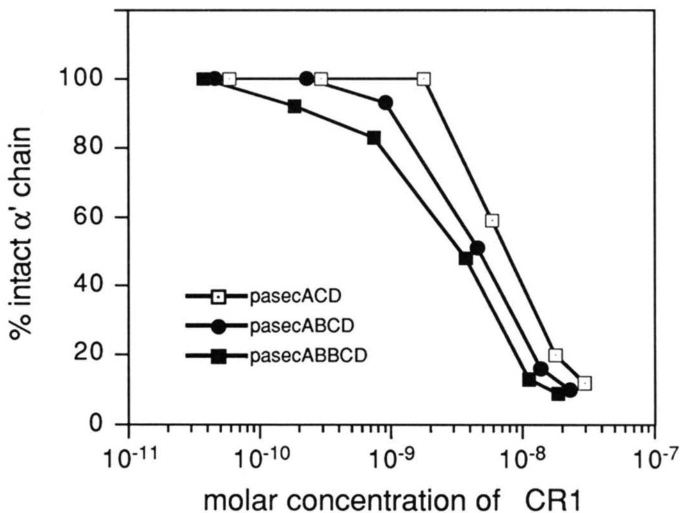


Figure 5. Cofactor activity of soluble rCR1. Cleavage of the α′ chain of C3b was measured in the presence of increasing amounts of soluble rCR1 derived from COS cells transfected with pasecABBCD, pasecABCD, or pasecACD.

ities for C3b-coated targets, the rCR1 variants were used to inhibit the uptake of ^{125}I-C3b dimer by erythrocyte CR1. The concentrations required for 50% inhibition reflected the relative affinities for either the ligand or the cell bound receptor. In the experiment shown in Figure 6, 10 nM of unlabeled C3b dimer was required for 50% inhibition of the interaction between ^{125}I-C3b dimer and E CR1. Consistent with the results of Weisman et al. (6), the interaction of the receptor with monomeric C3b was much weaker, requiring 1 μM, or 100-fold more of this ligand to achieve similar inhibition (Fig. 6). The low affinity of this monomeric interaction suggested that cross-linking of two discrete molecules of soluble rCR1 by the dimeric C3b ligand would not be favored under these conditions and that occupancy of two intramolecular binding sites was necessary for effective competition. This divalent interaction was ascertained by the requirement of 10 and 100 nM of the pasecABCD- and the pasecACD-derived rCR1, respectively, for 50% inhibition. Interestingly, only 1 nM of the pasecABBCD-derived rCR1 having three C3b binding sites was needed for a similar effect (Fig. 6); and the stoichiometric ratio of this interaction was two molecules of C3b dimer per molecule of CR1. Thus the soluble rCR1 forms with one, two or three C3b binding sites differed in their affinities for dimeric C3b but not for the monomeric form of this ligand that had been used as substrate for factor I cleavage (Fig. 5).

Inhibition of alternative and classical pathway convertases. The capacities of the soluble rCR1 variants to block the alternative and classical pathway convertases were compared by measurement of the C3a and C5a released on incubation of human serum with zymosan or aggregated IgG. Although only 1 to 2 nM of the pasecABBCD or the pasecABCD-derived rCR1 were necessary to achieve 50% inhibition of both the alternative pathway C3 and C5 convertases, 30-fold more of the pasecACD rCR1 was required for similar effects (Figs. 7*A* and 8*A*). This is consistent with the above predictions that the rCR1 variants would have different affinities for the C3b homodimers in the C5 convertases (25). That the same effect was seen with the C3 convertase indicated the presence of multiple molecules of C3b in association with the properdin-stabilized C3bBb complex on the zymosan. In contrast, large differences were not observed among the rCR1 variants in their capacities to inhibit the classical pathway C3 and C5 convertase (Figs. 7*B* and 8*B*), indicating that they had similar affinities for the C4b molecules within the C4bC2a complex or the C4b/C3b

Downloaded from http://journals.aai.org/jimmunol/article-pdf/146/2/656/1050117/656.pdf by guest on 26 April 2025

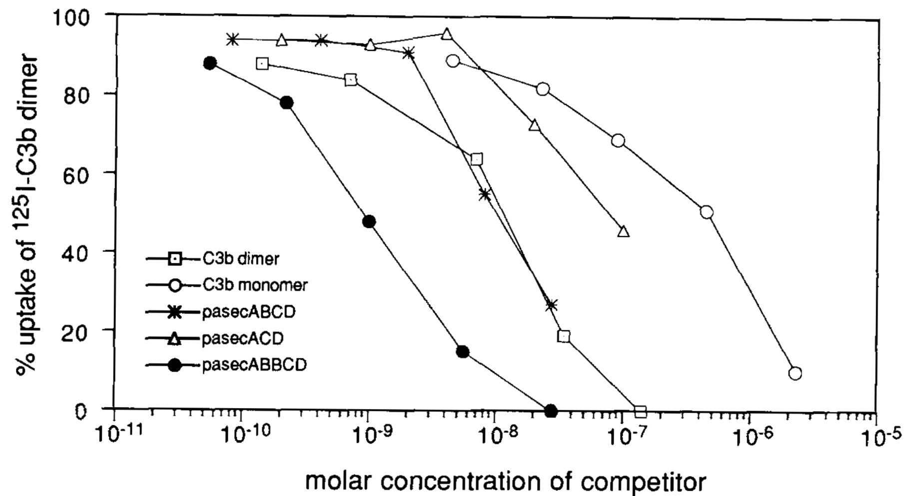


Figure 6. Inhibition of ^{125}I-C3b dimer uptake on erythrocytes by rCR1. E-bound ligand was measured in the presence of increasing concentrations of C3b dimer, C3b monomer, and rCR1 derived from COS cells transfected with pasecABBCD, pasecABCD, or pasecACD.

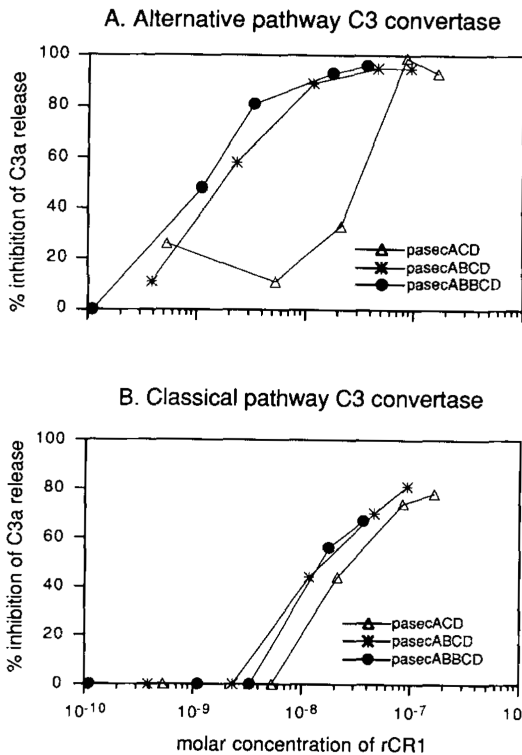


Figure 7. Inhibition of the alternative (*A*) and classical (*B*) C3 convertases by soluble rCR1 purified from COS cells transfected with the different plasmids encoding the CR1 variants.

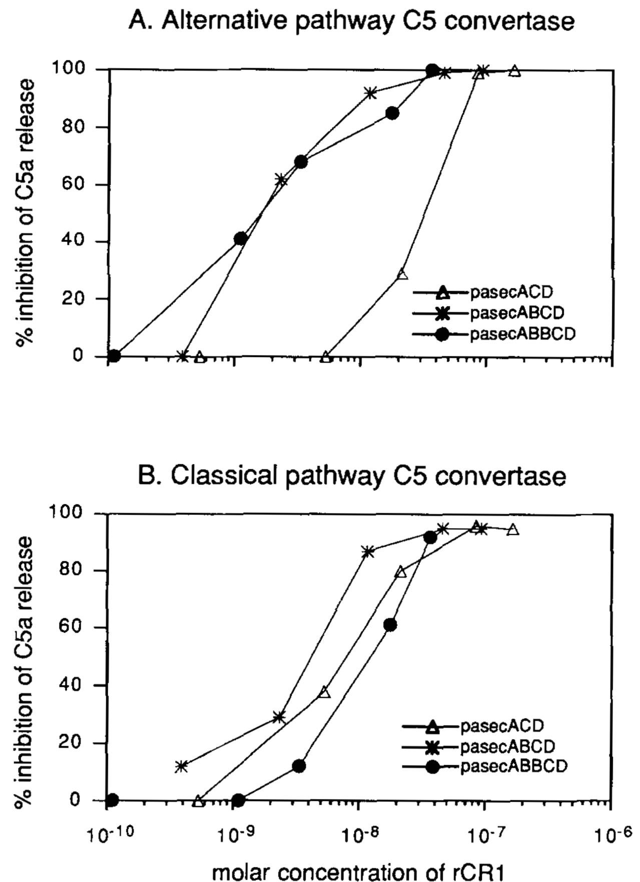


Figure 8. Inhibition of the alternative (*A*) and classical (*B*) C5 convertases by soluble rCR1 purified from COS cells transfected with the different plasmids encoding the CR1 variants.

heterodimers of the C5 convertases (26). Furthermore, except for the pasecACD-derived molecule, 2- to 10-fold more rCR1 was necessary to inhibit the enzymes of the classical relative to the alternative pathway, perhaps reflecting lower affinities of rCR1 for the C4b-containing convertases.

DISCUSSION

That each polymorphic variant of human CR1 is encoded by a different number of LHR is predicted by the ~1.3 kb differences in the transcripts associated with each allotype (14, 15). The parallels between the homologies in the coding regions and the homologies in the corresponding noncoding regions of the different LHR of the CR1 gene enabled us to predict the coding sequences based on a restriction map of the genomic clones (8, 9, 16). Thus the fifth LHR in the S allele with a 5′ half that resembled LHR-B and a 3′ half that resembled LHR-A was expected to encode a third binding site for C3b in the S allotype (16). Inasmuch as *Eco*RV was the only restriction enzyme among 19 others that revealed a RFLP associated with the F′ allotype (14), a deletion in this allele apparently also involved a highly homologous region. In this study, the 18-kb *Eco*RV fragment associated with individuals who expressed the F′ allotype (14) hybridized with a combination of exon- and intron-derived probes that was specific for LHR-C (Figs. 1 and 2), a pattern consistent only with the deletion of LHR-B or an equiva-

Downloaded from http://journals.aai.org/jimmunol/article-pdf/146/2/656/1050117/656.pdf by guest on 26 April 2025

lent region. The resulting F′ allotype was thus predicted to contain only three repeating domains, LHR-A, -C, and -D, and one binding site each for C4b and C3b.

CR1 is unique among the members of the supergene family of C3 and C4 binding proteins in having a tandem array of separate binding sites for its ligands. That the presence of multiple C3b binding sites may increase the capacity of CR1 to recognize the C3 homodimers of the alternative pathway C3 and C5 convertases prompted a functional comparison of rCR1 that differed in their numbers of LHR-B. We used an assay first described by Weisman et al. (6) in which C3b ligands and soluble rCR1 variants were used to compete for uptake of radiolabeled C3b dimers by E CR1. In our study, the amounts of monomeric or dimeric C3b and pasecABCD-derived rCR1 necessary for 50% inhibition of binding of radiolabeled dimeric C3b to E (Fig. 6) were very similar to those in the earlier report (6). Moreover, the affinity of the purified rCR1 for dimeric C3b increased 10 fold with the addition of each C3b binding site, resulting in a 100 fold difference between rCR1 molecules derived from pasecACD or pasecABBCD (Fig. 6). Consistent with the previous observation that the C4b binding site in LHR-A retained low affinity for C3b (9), the pasecACD-derived rCR1 utilizing both LHR-A and -C was more effective than the C3b monomers in blocking the uptake of radiolabeled dimer (Fig. 6). This interaction, however, was not as effective as that with the pasecABCD-derived protein that had two sites specific for C3b. The higher affinity observed for the pasecABBCD-derived rCR1 relative to the pasecABCD-derived molecule similarly suggested engagement of two pairs of binding sites per CR1 molecule (Fig. 6), and confirmed the previous hypothesis that multivalent interaction was favored by such a tandem array of LHR (9, 16). Since the affinity of CR1 for monomeric C3b is relatively low (Fig. 6), the lack of gross differences in the cofactor capacities of the different rCR1 forms in this study (Fig. 5) and that of Seya et al. (18) indicated that the conditions used did not favor simultaneous occupancy of more than one active site by monomeric ligands.

Differences in the effectiveness of the soluble rCR1 variants to block the alternative pathway C3 and C5 convertases were predicted from their differential affinities for the C3b homodimers (25). Indeed, in this assay, the rCR1 variants having two or more C3b binding sites were at least 30-fold more efficient than that which had only one site (Figs. 7*A* and 8*A*). However, the pasecABBCD-derived rCR1 is not more effective than that derived from pasecABCD (Figs. 7*A* and 8*A*) although its binding capacity is much higher in a fluid phase assay (Fig. 6). Thus its full activity in this assay may be restricted by the topographical distribution of the convertase sites on the activating surface. Since all three variants studied had one site for C4b and retained at least one adjacent site for C3b, their comparable capacities to inhibit the classical pathway convertases were anticipated (Figs. 7*B* and 8*B*). Our findings are consistent with those of Seya et al. (18) with respect to the classical C3 convertase but differ with respect to the alternative C3 convertase. This may be explained by the previous use of nickel-stabilized fluid phase alternative pathway convertases (18) which contained only one molecule of C3b per complex (27) and would not be distinguished by the different CR1 variants.

That CR1 variants having multiple C3b binding sites may be more effective in the recognition of the C3b dimers deposited during activation of the alternative pathway suggest that the gene duplication events associated with such functional improvements may be selectively preserved. For possible therapeutic applications in vivo (6), a molecule with two adjacent C3b binding sites represents a more effective inhibitor of complement activation by the alternative pathway. Whether the length of the CR1 molecule determines the binding to immune complexes cannot be directly extrapolated from our data because the efficiency of uptake by CR1 can be enhanced by the clustering of this receptor on E (28, 29). However, individuals with low CR1 numbers may have fewer clusters of this receptor as well as fewer receptor molecules per cluster (28). Thus the combined presence of the F′ allotype and low amounts of CR1 in SLE patients may result in fewer total C3b and C4b binding sites and a decreased efficiency in the clearance of immune complexes from the circulation (12, 17, 30–32). The slightly lower affinities of rCR1 observed for the classical pathway convertases (Figs. 7 and 8) (6) indicate that the relative amounts of C4b and C3b deposited on soluble immune complexes may be critical to the CR1-dependent uptake and processing. The absence of preclustered CR1 in neutrophils under some conditions (33) suggests that mechanisms that induce receptor aggregation may be essential for the triggering of biologic reactions in nucleated cells (2, 3). A shorter CR1 allotype may further decrease the efficiencies of such interactions and lead to impairment of receptor-mediated cellular responses at tissue inflammatory sites.

Acknowledgments. The authors thank Drs. Anne Nicholson-Weller and Lloyd Klickstein for helpful discussions.

REFERENCES

1. **Ahearn, J. M., and D. T. Fearon.** 1989. Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21). *Adv. Immunol. 46:183.*
2. **Daha, M. R., A. C. Bloem, and R. E. Ballieux.** 1984. Immunoglobulin production by human peripheral lymphocytes induced by anti-C3 receptor antibodies. *J. Immunol. 132:1197.*
3. **Bacle, F., N. Haeffner-Cavaillon, M. Laude, C. Couturier, and M. D. Kazatchkine.** 1990. Induction of IL-1 release through stimulation of the C3b/C4b complement receptor type one (CR1, CD35) on human monocytes. *J. Immunol. 144:147.*
4. **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.*
5. **Iida, K., and V. Nussenzweig.** 1981. Complement receptor is an inhibitor of the complement cascade. *J. Exp. Med. 153:1138.*
6. **Weisman, H. F., T. Bartow, M. K. Leppo, H. C. Marsh, Jr., G. R. Carson, M. F. Concino, M. P. Boyle, K. H. Roux, M. L. Weisfeldt, and D. T. Fearon.** 1990. Soluble human complement receptor type 1: an in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science 249:146.*
7. **Campbell, R. D., S. K. A. Law, K. B. M. Reid, and R. B. Sim.** 1988. Structure, organization, and regulation of the complement genes. *Annu. Rev. Immunol. 6:161.*
8. **Klickstein, L. B., W. W. Wong, J. A. Smith, J. H. Weis, J. G. Wilson, and D. T. Fearon.** 1987. Human C3b/C4b receptor (CR1): demonstration of long homologous repeating domains that are composed of the short consensus repeats characteristic of C3/C4 binding proteins. *J. Exp. Med. 165:1095.*
9. **Klickstein, L. B., T. J. Bartow, V. Miletic, L. D. Rabson, J. A. Smith, and D. T. Fearon.** 1988. Identification of distinct C3b and C4b recognition sites in the human C3b/C4b receptor (CR1, CD35) by deletion mutagenesis. *J. Exp. Med. 168:1699.*
10. **Wong, W. W., J. G. Wilson, and D. T. Fearon.** 1983. Genetic regulation of a structural polymorphism of human C3b receptor. *J. Clin. Invest. 72:685.*

Downloaded from http://journals.aai.org/jimmunol/article-pdf/146/2/656/1050117/656.pdf by guest on 26 April 2025

11. **Dykman, T. R., J. L. Cole, K. Iida, and J. P. Atkinson.** 1983. Polymorphism of human erythrocyte C3b/C4b receptor. *Proc. Natl. Acad. Sci. USA 80:1698.*
12. **Dykman, T. R., J. A. Hatch, and J. P. Atkinson.** 1984. Polymorphism of the human C3b/C4b receptor: identification of a third allele and analysis of receptor phenotypes in families and patients with systemic lupus erythematosus. *J. Exp. Med. 159:691.*
13. **Dykman, T. R., J. A. Hatch, M. S. Aqua, and J. P. Atkinson.** 1985. Polymorphism of the C3b/C4b (CR1) receptor: characterization of a fourth allele. *J. Immunol. 134:1787.*
14. **Wong, W. W., C. A. Kennedy, E. T. Bonaccio, J. G. Wilson, L. B. Klickstein, J. H. Weis, and D. T. Fearon.** 1986. Analysis of multiple restriction fragment length polymorphism of the gene for the human complement receptor type 1: duplication of genomic sequences occurs in association with a high molecular mass receptor allotype. *J. Exp. Med. 164:1531.*
15. **Holers, V. M., D. D. Chaplin, J. F. Leykam, B. A. Gruner, V. Kumar, and J. P. Atkinson.** 1987. Human CR1 mRNA polymorphism correlates with the CR1 allelic molecular weight polymorphism. *Proc. Natl. Acad. Sci. USA 84:2459.*
16. **Wong, W. W., J. M. Cahill, M. D. Rosen, C. A. Kennedy, E. T. Bonaccio, M. J. Morris, J. G. Wilson, L. B. Klickstein, and D. T. Fearon.** 1989. Structure of the human CR1 gene: molecular basis of the structural and quantitative polymorphisms and identification of a new CR1-like allele. *J. Exp. Med. 169:847.*
17. **Van Dyne, S., V. M. Holers, D. M. Lublin, and J. P. Atkinson.** 1987. The polymorphism of the C3b/C4b receptor in the normal population and in patients with systemic lupus erythematosus. *Clin. Exp. Immunol. 68:570.*
18. **Seya, T., V. M. Holers, and J. P. Atkinson.** 1985. Purification and functional analysis of the polymorphic variants of the C3b/C4b receptor (CR1) and comparison with H, C4b-binding protein (C4bp), and decay accelerating factor (DAF). *J. Immunol. 135:2661.*
19. **Seed, B.** 1987. An LFA-3 cDNA encodes a phospholipid-linked membrane protein homologous to its receptor CD2. *Nature 329:840.*
20. **Ausubel, F. M., R. Brant, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman and K. Struhl, eds.** 1987. *Current Protocols in Molecular Biology.* John Wiley & Sons/Greene Assoc., New York.
21. **Wong, W. W., R. M. Jack, J. A. Smith, C. A. Kennedy, and D. T. Fearon.** 1985. Rapid purification of the human C3b/C4b receptor (CR1) by monoclonal antibody affinity chromatography. *J. Immunol. Methods. 82:303.*
22. **Tack, B. F., and J. W. Prahl.** 1976. Third component of human complement: purification from plasma and physicochemical characterization. *Biochemistry 15:4512.*
23. **Fearon, D. T.** 1977. Purification of C3b inactivator and demonstration of its two polypeptide chain structure. *J. Immunol. 119:1248.*
24. **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.*
25. **Kinoshita, T., Y. Takata, H. Kozono, J. Takeda, K. Hong, and K. Inoue.** 1988. C5 convertase of the alternative complement pathway: covalent linkage between two C3b molecules within the trimolecular complex enzyme. *J. Immunol. 141:3895.*
26. **Takata, Y., T. Kinoshita, H. Kozono, J. Takeda, E. Tanaka, K. Hong, and K. Inoue.** 1987. Covalent association of C3b with C4b within C5 convertase of the classical complement pathway. *J. Exp. Med. 165:1494.*
27. **Fishelson, Z., M. K. Pangburn, and H. J. Muller-Eberhard.** 1984. Characterization of the initial C3 convertase of the alternative pathway of human complement. *J. Immunol. 132:1430.*
28. **Paccaud, J. P., J. L. Carpentier, and J. A. Schifferli.** 1988. Direct evidence for the clustered nature of complement receptor type 1 on the erythrocyte membrane. *J. Immunol. 141:3889.*
29. **Chevalier, J., and M. D. Kazatchkine.** 1989. Distribution in clusters of complement receptor type 1 (CR1) on human erythrocytes. *J. Immunol. 142:2031.*
30. **Wilson, J. G., N. A. Andriopoulos, and D. T. Fearon.** 1987. CR1 and the cell membrane proteins that bind C3 and C4: a basic and clinical review. *Immunol. Res. 6:192.*
31. **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. 2:493.*
32. **Schifferli, J. A., Y. C. Ng, J. Estreicher, and M. J. Walport.** 1988. The clearance of tetanus toxoid/anti-tetanus toxoid immune complexes from the circulation of humans: complement- and erythrocyte complement receptor 1- dependent mechanisms *J. Immunol. 140:899.*
33. **Paccaud, J. P., J. L. Carpentier, and J. A. Schifferli.** 1990. Difference in the clustering of complement receptor type 1 (CR1) on polymorphonuclear leukocytes and erythrocytes: effect on immune adherence. *Eur. J. Immunol. 20:283.*

Downloaded from http://journals.aai.org/jimmunol/article-pdf/146/2/656/1050117/656.pdf by guest on 26 April 2025