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Authors

Lowell, CA
Klickstein, LB
Carter, RH
[et al.](#)

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MAPPING OF THE EPSTEIN-BARR VIRUS AND C3dg BINDING SITES TO A COMMON DOMAIN ON COMPLEMENT RECEPTOR TYPE 2

BY CLIFFORD A. LOWELL, LLOYD B. KLICKSTEIN,
ROBERT H. CARTER, JACQUELINE A. MITCHELL,
DOUGLAS T. FEARON, AND JOSEPH M. AHEARN

*From the Division of Molecular and Clinical Rheumatology, Department of Medicine,
The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

Epstein-Barr Virus (EBV), a herpesvirus that infects most humans, causes infectious mononucleosis and has been implicated in the pathogenesis of several human malignancies including Burkitt's lymphoma, X-linked lymphoproliferative syndrome, and nasopharyngeal carcinoma (1-3). Immunocompromised patients undergoing immunosuppressive therapy or having the acquired immune deficiency syndrome (AIDS) are subject to EBV-induced B cell lymphomas, because of impaired T cell regulation of the proliferation of latently infected B lymphocytes (4). EBV also has been detected in Reed-Sternberg cells of patients with Hodgkin's lymphoma (5). In vitro, this virus is unique in its ability to immortalize efficiently human B cells (6).

EBV selectively infects B lymphocytes and some epithelial cells via the specific interaction between the major envelope protein of the virus gp350/220 and a cellular receptor, human complement receptor type 2 (CR2; CD21)¹ (7-9). The importance of this receptor in determining the tissue tropism of EBV is indicated by the finding that expression of CR2 in murine L cells renders them susceptible to viral infection (10). CR2 is a phosphoprotein (11) that normally functions to regulate B cell proliferation by interaction with its natural ligand, the C3dg fragment of the third component of the complement system (12-14). The receptor is composed of an extracellular domain made up entirely of 15 or 16 tandem short consensus repeats (SCRs) of ~60 amino acids each, a single transmembrane region and a 34 amino acid cytoplasmic tail (15, 16). Each SCR has four invariant cysteine residues that are probably disulfide bonded in a cys-1 to cys-3, cys-2 to cys-4 pattern forming a triple loop structure (17, 18). The tandem alignment of SCRs, each having dimensions of 38 × 30 Å, yields an extended beads-on-a-string appearance when other proteins having this structural motif are examined by electron microscopy (19, 20).

The presence of SCRs indicates that CR2 is a member of a family of mammalian proteins that contain a variable number of SCRs, ranging from two for the α subunit

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Address correspondence to Joseph M. Ahearn, Department of Medicine, The Johns Hopkins School of Medicine, 725 N. Wolfe Street, 617 Hunterian, Baltimore, MD 21205.

¹ Abbreviations used in this paper: CR2, complement receptor type 2; SCR, short consensus repeats.

of the IL-2R to 30 for complement receptor type 1 (CR1; CD35) (reviewed in reference 21). Proteins in this family are involved in the processes of inflammation, immune response, and tissue repair and include several plasma and cellular components of the complement system, factor XIIIb of the coagulation cascade, the IL-2R, and the cell adhesion molecules ELAM-1 and the lymphocyte homing receptor (21-23). Analysis of CR1 and IL-2R by deletional and site-directed mutagenesis has indicated that each of three binding sites in CR1 and the single site in the IL-2R require the function of at least two SCRs (24, 25).

The SCRs comprising the binding sites for EBV and C3dg on CR2 are not known but may be identical. An anti-receptor mAb, OKB-7, blocks uptake of both ligands, as do multimeric forms of a peptide corresponding to the NH₂ terminus of gp350/220 that is homologous to a CR2-binding site in C3dg (26, 27). The viral and natural ligand binding sites of two other receptors may also be proximate or identical; mAbs that block uptake by CD4 and ICAM-1 of HIV and rhinovirus, respectively, also inhibit the interaction of these receptors with their natural ligands, the class II MHC antigen and LFA-1 (28-30). Moreover, the residues in CD4 required for binding of gp120 may also be necessary for interaction with MHC class II (31). Therefore, it has been suggested that certain viruses may have adapted to recognize the natural ligand binding sites of cellular receptors (32).

To determine whether EBV and C3dg use common structural elements of CR2 for binding, we constructed and functionally assessed a series of CR2 deletion mutants and chimeric receptors containing SCRs, or portions thereof, from CR2 and CR1. We have concluded that the NH₂-terminal two SCRs of CR2 are required for the binding of both ligands.

Materials and Methods

Plasmids. The isolation of overlapping CR2 cDNA clones λ 1.3 (10) and λ 4.11 (16) has been described previously. The λ 1.3 Eco RI insert was subcloned into pUC18 to generate pCR2 1.3 and the λ 4.11 Eco RI insert was subcloned into pBR322 to generate pCR2 4.11. These plasmids were digested with Eco RI/Nsi I and Nsi I/Cla I, respectively, and the appropriate CR2-containing fragments were ligated with Eco RI/Cla I-digested pBluescript KS+ (Stratagene, La Jolla, CA) to generate, pBSCR2.1 in which the full-length CR2 cDNA is inserted in an Eco RI (5') to Cla I (3') orientation. In a similar manner, pCR2 1.3 and pCR2 4.11 were digested with Xba I/Nsi I and Nsi I/Sph I, respectively, and these CR2 fragments were ligated with Xba I/Sph I-digested pUC19 to generate pCR2.1 in which the full-length CR2 cDNA clone is in an Xba I (5') to Sph I (3') orientation. Clone pBSCR2X was created by removing the insert from pBSCR2.1 with Xba I and ligating with Xba I-digested pBSKS⁺, followed by isolation of a clone in which the orientation of the CR2 cDNA insert was reversed.

Deletion Mutants. Plasmid pCR2 XB was generated by cleavage of pCR2.1 with Xho I and Bsm I, followed by modification with T4 DNA polymerase in the presence of dNTPs (New England Biolabs, Beverly, MA). The modified 5.9-kb fragment containing CR2 sequences 5' of the Xho I site and 3' of the Bsm I site was ligated with Sph I linkers containing the nucleotides GGCATGCC, digested with Sph I, and self-ligated. The sequence generated at the deletion site (CTC GAG GCA TGC CAG) resulted in a valine (GCA) for glutamic acid (GAA) substitution, while otherwise restoring the reading frame that joined the fourth cysteine of SCR-2 with the first cysteine of SCR-7.

Plasmid pCR2 NN was generated by digestion of pCR2.1 with Nde I and religation of the 5.8-kb fragment to itself. The Nde I sites are conserved within the homologous SCR-5

and SCR-9; therefore, deletion of the internal Nde I fragment followed by religation restores the correct reading frame with appropriate spacing of cysteine residues.

Plasmid pCR2 EK was constructed by subcloning from pBSCR2X the Xho I, made blunt ended by filling in with Klenow enzyme (New England Biolabs), to Hind III fragment containing SCR-3 through SCR-15 into pUC19 cleaved with Eco RI (blunt ended) and Hind III. This subclone was digested with Eco RI and Kpn I, treated with T4 DNA polymerase, ligated with Sph I linkers (GGCATGCC), and digested with Sph I. The larger fragment containing CR2 sequences 5' to the Eco RI site and 3' to the Kpn I site was isolated, treated with T4 polymerase, and self-ligated. An internal CR2 fragment containing the Eco RI/Kpn I deletion was removed from this subclone by Cla I/Hind III digestion and ligated into these same sites in pCR2.1 to form pCR2 EK. The resulting sequence at the deletion junction, GGA TTG GCC CAG, introduces a single alanine codon, GCC, between the Eco RI and Kpn I sites.

Plasmid pCR2 NK was created by removing the Nsi I/Nde I fragment from pCR2 EK, which includes portions of SCR-1 through SCR-9, and replacing it with the Nsi I/Nde I fragment from pBSCR2 NN, which contains portions of SCR-1 through SCR-5.

Plasmid, pCR2 NP, was constructed by subcloning the Cla I/Pst I fragment containing SCR-6 through SCR-15 from pCR2.1 into pBSKS⁺. This subclone was digested completely with Nde I and partially with Pvu II, and the 3.8-kb fragment containing portions of SCR-6 through SCR-9 and SCR-14 SCR-15 was ligated to a synthetic double-stranded oligonucleotide containing the sequence 5' TAT GGA ACC ACG CTC ACT TAC ACA AG 3', which restored the correct reading frame and maintained inter-cysteine spacing without adding extra codons across the deletion site. The Cla I/Pst I fragment from the subclone containing the Nde I to Pvu II deletion was ligated into these same sites in pCR2.1 to form pCR2 NP.

Plasmid pCR2 NOP was constructed by removing the Nsi I/Nde I fragment, containing SCR-1 through SCR-9, from pCR2 NP and replacing it with the Nsi I/Nde I fragment, containing SCR-1 through SCR-5, from pBSCR2 NN.

Plasmid pBSCR2 PP was created by partial digestion of pBSCR2X with Pvu II, followed by self-ligation of the 4.4-kb fragment containing CR2 sequence 5' to the Pvu II site within SCR 3 and 3' to the Pvu II site within SCR 14. These two Pvu II sites are conserved within homologous SCRs, and self-ligation of this fragment restores the correct reading frame and maintains the inter-cysteine spacing across the Pvu II/Pvu II deletion.

Chimeric Mutants. Plasmids encoding receptors comprised of portions of both CR1 and CR2 were constructed using the full-length CR1 cDNA clone pBSABCD, or the mutants pBSAD and pBSCD, which were generated by fusion of LHR-A and LHR-D, and deletion of LHR-A and -B, respectively, from pBSABCD (24). The order of CR1 and CR2 used in the plasmid nomenclature reflects the 5' to 3' composition of the construct.

Plasmid pBSCR1/CR2 N was constructed by ligation of the 3.1-kb Nsi I/Xba I fragment of pBSCR2.1 to the 3.4-kb Nsi I/Xba I fragment of pBSABCD. This results in fusion of the Nsi I site in SCR-2 of CR1 to the Nsi I site in SCR-1 of CR2. The fusion occurs at the third cysteine residue in each SCR, resulting in natural restoration of the reading frame from CR1 to CR2 without addition of extra codons. The resulting chimera encodes 16 SCRs, one more than pBSCR2X.

Plasmid pBSCR1/CR2 EX was constructed by cleavage of pCR2.1 with Xho I, treatment with Klenow fragment, protection of internal Eco RI sites with Eco RI methylase (New England Biolabs), followed by ligation with the Eco RI linker, CGGAATTCCG, and digestion with Eco RI and Xba I. This produced a 2.9-kb Eco RI (5')-Xba I (3') fragment that was ligated with both a 520-bp Eco RI fragment containing SCR-1 and SCR-2 from pBSABCD and pBSKS⁺ digested with Eco RI and Xba I. This construct was partially digested with Eco RI, treated with Klenow fragment, and self-ligated. A clone containing a modified Eco RI site at the CR1/CR2 fusion junction was isolated in which the signal peptide and SCR-1 and SCR-2 of CR1 are spliced to SCR-3 of CR2. The resulting sequence at the fusion junction AAT TCC GTC encodes the amino acids asparagine-serine-valine, that are not present in either CR1 or CR2.

Plasmid pBSCR2/CR1 N was created by digestion of pBSAD with Not I followed by par-

tial digestion with Nsi I. The 3.7-kb fragment containing CR1 sequences 3' to the Nsi I site within SCR-2 was ligated to pBSCR2X that had been digested with Nsi I and Not I. This chimera is the reciprocal of the Nsi I fusion used in pBSCR1/CR2 N, results in natural restoration of the reading frame from CR2 to CR1, and is one SCR shorter than pBSAD.

Plasmid pBSCR2/CR1 SE was constructed by digestion of pCR2.1 with Ssp I, followed by treatment with Eco RI methylase, ligation of an Eco RI linker containing the sequence CCGGAATTCCGG, and digestion with Eco RI and Sal I. The 350-bp fragment containing CR2 sequence 5' of the modified Ssp I site was ligated to pBSCD digested with Sal I and Eco RI to fuse SCR-1 of CR2 to SCR-17 of CR1 in a manner that restores the reading frame and results in a chimera that is one SCR shorter than native pBSCD. The sequence at the fusion junction TGT GAA TCC GGA ATT CCT contains two codons for serine and glycine which are not present in either CR1 or CR2.

Plasmid pBSCR2/CR1 XE was constructed by digestion of pCR2.1 with Xho I, followed by treatment with Klenow fragment, methylation of Eco RI sites, ligation of an Eco RI linker containing the sequence CCGGAATTCCGG, and digestion with Eco RI and Sal I. The 550-bp Sal I/Eco RI fragment containing CR2 sequences 5' of the modified Xho I site was ligated with both the 3.6-kb Eco RI/Xba I fragment from pBSCD and pBSKS⁺ digested with Sal I/Xba I to result in fusion of SCR-1 and -2 of CR2 to SCR-17 of CR1 while maintaining the reading frame. The sequence at the fusion junction CTC GAC GGA ATT CCT contains a single glycine residue in place of the arginine normally present in CR1.

Plasmid pBSCR2/CR1/CR2 KON was constructed by subcloning the Sal I/Bam HI fragment, containing SCR-1 through SCR-9, from pBSCR2X into a pBSKS⁺ plasmid in which the Kpn I site had been removed from the polylinker through sequential digestion with Kpn I and T4 DNA polymerase followed by self-ligation. The subclone was digested with Kpn I and Nsi I and the larger fragment was ligated with a synthetic double-stranded oligonucleotide that contained the sequence GT ACC GTG ATA AGG TAC AGT TGC CGC CCT GGT TAT TCC GGA AGA CCG TTT TCT ATC ATC TGC T. This resulted in replacement of the sequences encoding amino acids between cysteines 2 and 3 of SCR-1 of CR2 with those between cysteines 2 and 3 of SCR-1 of CR1, such that the sequence CSGTFR-LIGEKSLLC was converted to CRPGYSGRPFSIIC.

Deletion mutants and chimeras were confirmed by restriction mapping and dideoxynucleotide sequencing of double-stranded plasmids or M13mp18 subclones (33, 34). Enzymes were used as described by the manufacturer. Bacterial strain DH5 α (Bethesda Research Laboratories, Gaithersburg, MD) was used for propagation of the above plasmids.

The complete inserts that encoded deletion and chimeric mutant receptors were removed from bacterial vectors with either Xba I alone or Not I and Xho I, and cloned into these sites in the eukaryotic expression vector CDM8 (35), which was propagated in bacterial strain DK1/P3 (24). The CDM8-containing constructs are preceded by pi in the nomenclature used here.

Antibodies. HB-5 (IgG2a), OKB-7 (IgG2a) (Ortho Pharmaceuticals, Raritan, NJ), and B2 (IgM) (Coulter Immunology, Hialeah, FL) are mouse mAbs specific for human CR2 (8, 36) YZ-1 is an anti-CR1 mouse IgG1 mAb (11). Mouse mAb 72A1 is an IgG that recognizes the EBV gp350/220 glycoprotein (37). UPC-10 is an IgG2a mouse mAb that recognizes levan and inulin (Bionetics Laboratory Products, Charleston, SC). Fluorescein-conjugated or Texas Red-conjugated goat F(ab')₂ anti-mouse IgG and goat F(ab')₂ anti-mouse IgM were purchased (Jackson ImmunoResearch Laboratories, West Grove, PA). Goat F(ab')₂ anti-mouse IgG or anti-mouse IgM were radiolabeled with ¹²⁵I to a specific activity of 5 FT32 $\frac{1}{4}$ 10⁹ cpm/ μ g using the iodo-bead method (Pierce Chemical Co., Rockford, IL).

Polymerized C3dg, gp350/220, and EBV. C3dg was prepared from aged human serum by chromatography on DEAE-Sephacel (Pharmacia Fine Chemicals, Piscataway, NJ) followed by gel filtration through Sephacryl S-200 HR (Pharmacia Fine Chemicals) (13). C3dg was polymerized with an 80-fold molar excess of glutaraldehyde and polymers were isolated by chromatography through Sephacryl S-200. Analysis of polymerized C3dg (pC3dg) by sucrose gradient ultracentrifugation demonstrated an average molecular weight of 450,000, consistent with polymers ranging from tetramers to 20-mers. Polymerized C3dg was conjugated

to FITC to a FITC:pC3dg molar ratio of four. Polymerized C3dg was radiolabeled with ^{125}I to a specific activity of 2×10^6 cpm/ μg using the iodo-bead method.

Purified recombinant gp350/220 (38) (provided by N. Cooper and G. Nemerow, Research Institute of Scripps Clinic, La Jolla, CA) was prepared by immunoaffinity chromatography of culture supernatants of GH3 cells secreting a truncated, soluble form of the protein that lacks the transmembrane and cytoplasmic domains. The preparation, which contained approximately equal portions of gp350 and gp220, was ^{125}I labeled to a specific activity of 2×10^6 cpm.

The B95-8 strain of EBV was prepared as described (39), and used as either 1,000-fold concentrated culture supernatant or as dextran gradient-purified virus.

Transfection. Plasmids were transiently expressed by transfection into COS cells using the DEAE-dextran procedure (40). Murine L cells were stably transfected with pMT.CR2.neo.1 (10), or co-transfected with piCR2/CR1 XE and pSVneo.1 (which contains the neo gene driven by the HSV TK promoter; provided by L. Sanders, Department of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, MD) using the calcium phosphate procedure as described (41). Transfected cells were selected for 14 d in 400 $\mu\text{g}/\text{ml}$ of G418 (Gibco Laboratories, Grand Island, NY); resistant colonies were pooled and assayed for CR2 or CR2/CR1 XE expression.

L cells transfected with pMT.CR2.neo.1 or piCR2/CR1 XE and bearing relatively high numbers of recombinant receptors were obtained by indirectly staining with the mAbs HB-5 and YZ-1, respectively, and performing four (pMT.CR2.neo.1) or two (piCR2/CR1 XE) consecutive rounds of fluorescence activated cell sorting in which the brightest 3–12% of each population were recovered with each sort.

Immunofluorescence, Radioimmunoassay, and Immunoprecipitation. COS cells or L cells were harvested with PBS containing 1 mM EDTA and washed with PBS containing 0.1% BSA and 0.02% sodium azide (PBSA). Replicate samples of 5×10^4 to 5×10^5 cells in 0.1 ml of PBSA were sequentially incubated at 0°C for 30 min with 1.0 $\mu\text{g}/\text{ml}$ OKB-7, or with 4.0 $\mu\text{g}/\text{ml}$ of HB-5, YZ-1, UPC-10, or B2, washed, and incubated with second antibody labeled with FITC, Texas Red, or ^{125}I . Cells were assayed for ^{125}I or were observed for fluorescence by microscopy or flow cytometry utilizing a Coulter Epics model 752 counter and correcting for autofluorescence (42). Cells also were incubated with 5.0 $\mu\text{g}/\text{ml}$ FITC-pC3dg with or without preincubation with 40-fold molar excess of unlabeled pC3dg and assayed for fluorescence. Immunofluorescent analysis of the binding of EBV was performed as described (10).

Approximately 10^7 L cells or 10^6 COS cells transfected with recombinant plasmids were surface labeled with Iodo-Gen (Pierce Chemical Co.). Detergent lysates of labeled cells were sequentially immunoadsorbed with Sepharose-UPC-10 followed by either Sepharose-HB-5 or Sepharose-YZ-1. COS cells transfected with piCR2 PP, which reacts with OKB-7 only, were sequentially incubated with Sepharose-protein A (Sigma Chemical Co.), OKB-7, and Sepharose-protein A. Adsorbed proteins were eluted by boiling in 1% SDS and assessed by SDS-PAGE and autoradiography.

Quantitative Binding of ^{125}I -pC3dg and ^{125}I -gp350/220. Duplicate or triplicate samples of 5×10^5 L cells expressing wild-type CR2 or 10^6 L cells expressing CR2/CR1 XE, Raji cells, or untransfected L cells were incubated in 0.1 ml of PBSA with 0.1 $\mu\text{g}/\text{ml}$ ^{125}I -pC3dg or 0.25 $\mu\text{g}/\text{ml}$ ^{125}I -gp350/220 in the presence or absence of increasing concentrations of unlabeled ligand for 1 h at 0°C. Cells were centrifuged through 0.3 ml of a 1.5:1 mixture of dibutylphthalate/dinonylphthalate in 0.4 ml polypropylene microfuge tubes for 2 min at 8,000 rpm at room temperature. The tubes were cut and cell bound and free ^{125}I were determined.

Results

Expression and Analysis of CR2 Deletion Mutants and CR2/CR1 Chimeric Receptors. Expression of CR2 by transfected COS cells was assessed by measurement of the binding of EBV, polymerized C3dg (pC3dg), and three anti-CR2 mAbs. Polymerized C3dg was used for these studies because of the low affinity of CR2 for monomeric C3dg (43). mAb OKB-7, which inhibits uptake by CR2 of both pC3dg and EBV, and

mAbs HB-5 and B2, which do not, bound to 30, 33, and 39% of the transfected COS cells, respectively, as demonstrated by surface immunofluorescence (Fig. 1); these antibodies also bound specifically to CR2-transfected COS cells when assessed by RIA (Table I). The fraction of cells that stained with the mAbs was equivalent to that fluorescently labeled with FITC-pC3dg and EBV (Fig. 1). When CR2 transfected COS cells were stained simultaneously with FITC pC3dg and indirectly labeled Texas Red HB-5, there were no singly positive cells for either fluorochrome, indicating correspondence between recombinant CR2 antigen and ligand binding function. The membrane protein expressed by the COS cells transfected with the full length cDNA construct had a M_r of 145,000, when immunoprecipitates of surface-labeled cells were analyzed by SDS-PAGE, which is comparable to that of CR2

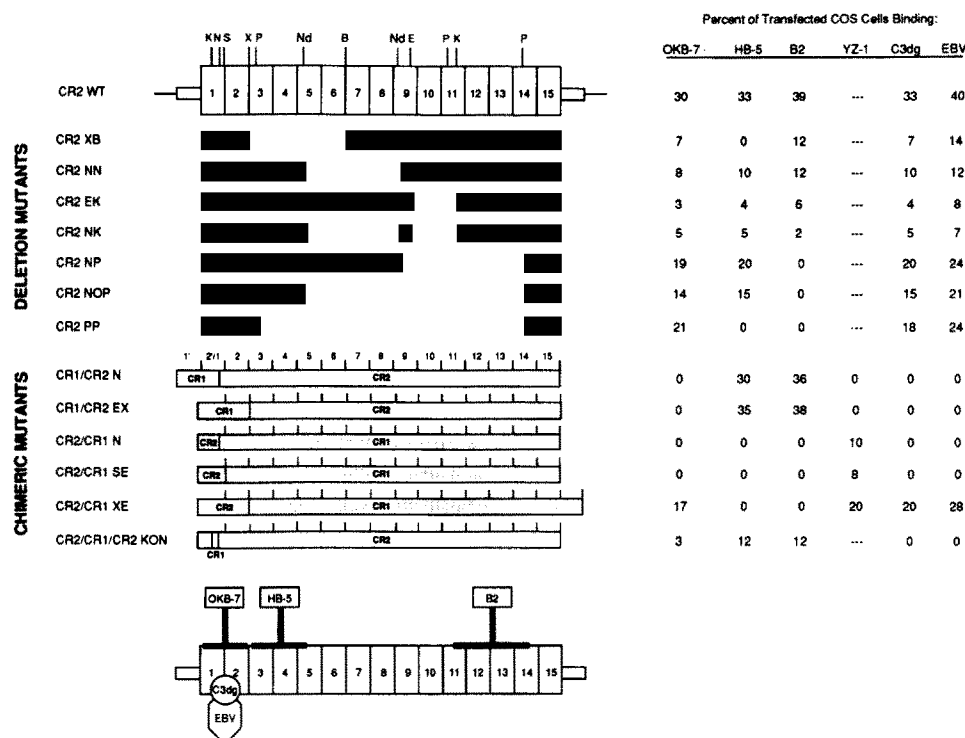


FIGURE 1. Binding of mAbs and ligands to deletion and chimeric mutants of CR2. Shown on the left are schematic representations of deletion and chimeric mutants beneath a partial restriction map of wild-type CR2 cDNA in which positions of the 15 SCRs are delineated. Restriction sites are abbreviated: B, Bsm I; E, Eco RI; K, Kpn I; N, Nsi I; Nd, Nde I; P, Pvu II; S, Ssp I; X, Xho I. The Kpn I and Ssp I sites indicated within SCR-1 are present as pairs of each site separated by ~20 bp each. The chimeric mutants are aligned with respect to numbering of CR2 SCRs. Shown to the right of each construct are the immunofluorescent analyses of the binding of the anti-CR2 mAbs, HB-5, OKB-7, and B2, the anti-CR1 mAb, YZ-1, and the CR2 ligands pC3dg and EBV, to COS cells expressing each mutant. The results are expressed as the percent of transfected COS cells that are immunofluorescently labeled with each mAb or CR2 ligand as determined by counting at least 250 cells. Absence of binding indicates no positives among at least 2,000 cells counted. Below is a model demonstrating the positions for the anti-CR2 mAb epitopes and the binding sites for EBV and pC3dg.

from previously characterized stably transfected L cells expressing a full-length CR2 construct (Fig. 2) (10).

A series of deletion mutants of recombinant CR2 were constructed in a manner that would maintain the proper spacing of the four conserved cysteines of each SCR to enable formation of the predicted disulfide bonds. This aim was achieved in some mutants by the use of conserved restriction sites residing in homologous SCRs, and in others by the addition of oligonucleotide linkers encoding additional amino acids. Thus, the deletion mutants vary in size by an integral number of SCRs, and the number of SCRs deleted ranges from a minimum of two for CR2 EK to a maximum of 11 for CR2 PP (Fig. 1). Immunoprecipitation of surface-labeled COS cell transfectants with anti-CR2 mAbs indicated that several deletion mutants directed synthesis of receptors of the predicted M_r : the CR2 PP deletion mutant which has four SCRs was $\sim 40,000 M_r$, and the CR2 NN mutant which has 12 SCRs was $\sim 107,000 M_r$ (Fig. 2). The CR2 PP mutant was well expressed as determined by immunofluorescence and RIA (Fig. 1; Table I).

Two deletion mutants did not bind HB-5: CR2 XB which lacks SCR-3 through SCR-6, and CR2 PP which has a large internal deletion affecting SCR-3 through SCR-14 (Fig. 1). The finding that HB-5 did bind to COS cells expressing the CR2 NN mutant, which lacks the COOH-terminal two-thirds of SCR-5 through the first third of SCR-9, localizes the epitope for this mAb to a region including SCR-3, SCR-4, and the first third of SCR-5.

Three deletion mutants did not bind B2: CR2 NP which lacks most of SCR-9, all of SCR-10 through SCR-13, and a portion of SCR-14; CR2 NOP which has a further deletion extending 5' to SCR-5; and the CR2 PP mutant having the largest deletion extending up through SCR-3 (Fig. 1). The capacity of COS cells expressing

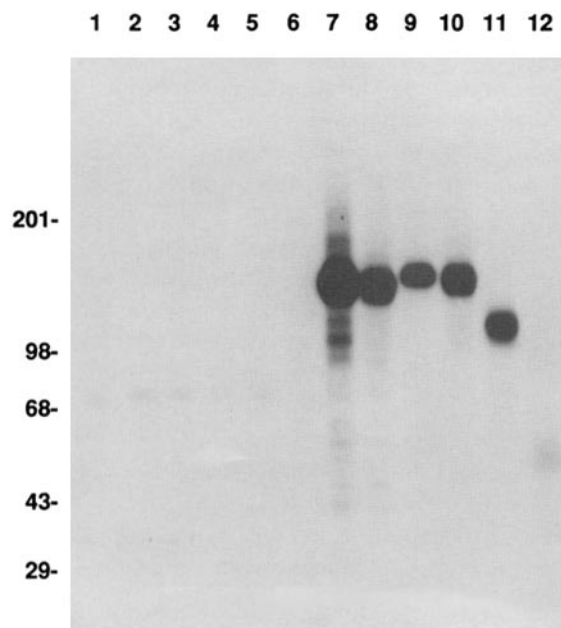


FIGURE 2. Comparison of recombinant wild-type CR2 with deletion and chimeric mutants. Detergent lysates of ^{125}I surface-labeled murine L cells stably expressing wild-type CR2 (lanes 1 and 7) and COS cells transfected with piCR2 (lanes 2 and 8), piCR2/CR1 XE (lanes 3 and 9), piCR1/CR2 N (lanes 4 and 10), piCR2 NN (lanes 5 and 11), and piCR2 PP (lanes 6 and 12), respectively, were immunoprecipitated with Sepharose-UPC-10 anti-levan (lanes 1-5), UPC-10 and protein A-Sepharose (lane 6), Sepharose-HB-5 anti-CR2 (lanes 7, 8, 10, 11), Sepharose-YZ-1 (lane 9), and OKB-7 anti-CR2 and protein A-Sepharose (lane 12), respectively. The eluates were subjected to SDS-PAGE under reducing conditions and autoradiography for 36 h. The diminished intensity of the CR2 PP mutant compared with other CR2 proteins was caused by less efficient immunoprecipitation with OKB-7 and immobilized protein A than with HB-5 directly coupled to sepharose.

TABLE I
Binding of Anti-CR2 or Anti-CR1 mAbs to COS Cell Transfectants

Construct	OKB7	HB-5	B2	YZ-1
Deletion mutants				
CR2 WT	9.2	12.7	6.6	
CR2 XB	8.0	0.9	4.3	
CR2 NN	13.4	25.4	6.4	
CR2 EK	3.9	4.7	3.1	
CR2 NK	3.4	2.0	3.4	
CR2 NP	5.5	7.5	0.9	
CR2 NOP	5.0	6.9	1.0	
CR2 PP	12.9	1.3	0.7	
Chimeras				
CR1/CR2 N	1.0	13.7	9.1	0.6
CR1/CR2 EX	1.2	21.0	8.0	0.9
CR2/CR1 N	1.6	1.0	1.6	11.8
CR2/CR1 SE	1.2	0.8	0.8	3.8
CR2/CR1 XE	5.5	1.5	1.7	5.0
CR2/CR1/CR2 KON	4.8	8.9	4.1	

Fold increase in binding of ^{125}I -labeled second antibody to transfected COS cells preincubated with specific antibody relative to COS cells preincubated with irrelevant antibody, or relative to COS cells transfected with vector alone. Data are from the same transfection experiment shown in Fig. 1; all constructs were analyzed in at least two separate transfections with comparable results.

either the CR2 NK mutant or the CR2 EK constructs to bind B2, which together replace SCR-12, SCR-13, and a portion of SCR-14, indicates that the epitope for B2 resides within these SCRs.

All deletion mutants bound OKB-7, placing its epitope in the most NH₂-terminal and/or COOH-terminal two SCRs of CR2 (Fig. 1). Furthermore, since no deletion mutants lacked these two regions, the presence of additional epitopes for OKB-7 cannot be excluded by this analysis. All deletion mutants also bound EBV and pC3dg, indicating that the binding sites for these ligands co-localize with the OKB-7 epitope, which is consistent with the capacity of OKB-7 to inhibit uptake by CR2 of EBV and C3dg.

Chimeric receptors composed of SCRs from CR2 and its homologue, CR1, were prepared to define further the sequences required to form binding sites for EBV, pC3dg, and OKB-7. As with the deletion mutants, chimeras were constructed in a manner that preserved the spacing of conserved cysteine residues, and the recombinant chimeric receptors varied in size by integral SCR units. Substitution of the cys-1 through cys-3 region of SCR-1 in the full-length CR2 construct with a segment of CR1 consisting of SCR-1 and the cys-1 through cys-3 portion of SCR-2 was achieved in the CR1/CR2 N chimera (Fig. 1). This receptor was expressed well by COS cells, as assessed by the binding of the mAbs HB-5 and B2 (Fig. 1; Table I), and by immunoprecipitation followed by SDS-PAGE, revealing a receptor with a M_r slightly larger than that of wild-type CR2, because of the presence of the additional SCR (Fig. 2). However, the CR1/CR2 N chimera lacked the OKB-7 epitope and was unable to bind EBV and pC3dg (Fig. 1). Analysis of the effects of the more extensive

substitution in the CR1/CR2 EX chimera in which SCR-1 and SCR-2 of CR2 were replaced with these SCRs of CR1 confirmed the conclusion that the NH₂-terminal region of CR2 was required for the formation of sites involved in the uptake of OKB-7, EBV and pC3dg (Fig. 1).

The roles of SCR-1 and SCR-2 of CR2 in the formation of these ligand binding sites were assessed by expressing in COS cells chimeric receptors that contained variable portions of these two SCRs linked to the NH₂ terminus of two deletion mutants of CR1. The CR1 constructs on which the CR2 substitutions were made contain either the NH₂-terminal seven SCRs spliced to the COOH-terminal nine SCRs or all of the COOH-terminal 16 SCRs, along with transmembrane and cytoplasmic regions of the receptor. Both of these CR1 constructs have been shown previously to be expressed well in COS cells (24). Replacement of the cys-1 to cys-3 region of SCR-1, or of the entire NH₂-terminal SCR of CR1 with the corresponding regions from CR2 in the CR2/CR1 N and CR2/CR1 SE chimeras did not form a binding site for EBV, pC3dg, or OKB-7, despite reasonable expression in COS cells, as indicated by the uptake of the anti-CR1 mAb YZ-1 (Fig. 1; Table I). This observation, when coupled with that of the absence of ligand binding functions in the CR1/CR2 N chimera, indicates that SCR-1 is necessary but not sufficient for these ligand binding sites in CR2. In the chimera CR2/CR1 XE, both SCR-1 and SCR-2 of CR2 are substituted for the NH₂-terminal SCRs of the CR1 construct to form a membrane protein composed of 16 SCRs that is slightly larger than the 15-SCR form of wild-type CR2 (Fig. 2). This chimera was capable of binding EBV, pC3dg, and OKB-7, indicating that the combination of the NH₂-terminal two SCRs of CR2 are sufficient to constitute binding sites for these three ligands. Staining of COS cells transfected with this chimera with FITC-pC3dg and indirectly labeled Texas Red YZ-1 revealed no singly positive cells for either fluorochrome, indicating correspondence between expression of the recombinant CR2/CR1 chimera and CR2 ligand binding function.

An attempt to discriminate among the structural requirements for these three ligands was made by substituting the cys-2 to cys-3 region of SCR-1 of the full-length CR2 construct with this region of SCR-1 of CR1 in the chimera, CR2/CR1/CR2 KON (Fig. 1). Substitution of this region was chosen because mutations within the cys-2 to cys-3 portion of the first SCR of the α subunit of IL-2R result in a 100-fold decrease in IL-2 binding affinity (25). The COS cells expressing the CR2/CR1/CR2 KON receptor did not bind EBV or pC3dg, despite demonstrating specific uptake of OKB-7 (Fig. 1; Table I). Thus, the EBV and pC3dg binding site(s) in CR2 require both SCR-1 and SCR-2, and cannot tolerate a substitution of an amino acid segment from a homologous position in CR1 that represents only 10% of the linear sequence of the two SCRs, distinguishing this site(s) from the epitope for OKB-7.

Comparison of the CR2/CR1 EX Chimera with Wild Type CR2 in Stably Transfected Murine L Cells. The analytic studies identifying SCR-1 and SCR-2 as the EBV/C3dg binding domain of CR2 were extended by quantitatively comparing ligand binding to L cells bearing either the CR2/CR1 XE chimera or wild-type CR2. Pools of L cells transfected with pMT.CR2.neo.1 (10) were selected by four rounds of fluorescence activated sorting with indirectly labeled HB-5, and L cells co-transfected with CR2/CR1 XE and pSV.neo.1 were selected by two rounds of sorting with indirectly labeled YZ-1. Replicate samples of cells from both lines were assayed for fluores-

cence by flow cytometry after labeling with the following mAbs and ligands: HB-5, OKB-7, YZ-1, or irrelevant mAb UPC-10 (anti-levan) followed by indirect staining with FITC-conjugated second antibody; FITC-pC3dg alone or in the presence of excess, unconjugated pC3dg; and EBV followed by the monoclonal anti-gp350/220, 72A1, and indirect staining with FITC-conjugated second antibody. All L cells expressing wild-type CR2 specifically bound both anti-CR2 mAbs, pC3dg and EBV, but not anti-CR1 mAb YZ-1 (Fig. 3). No binding of FITC-pC3dg was observed in the presence of excess unlabeled pC3dg and no binding of EBV was observed

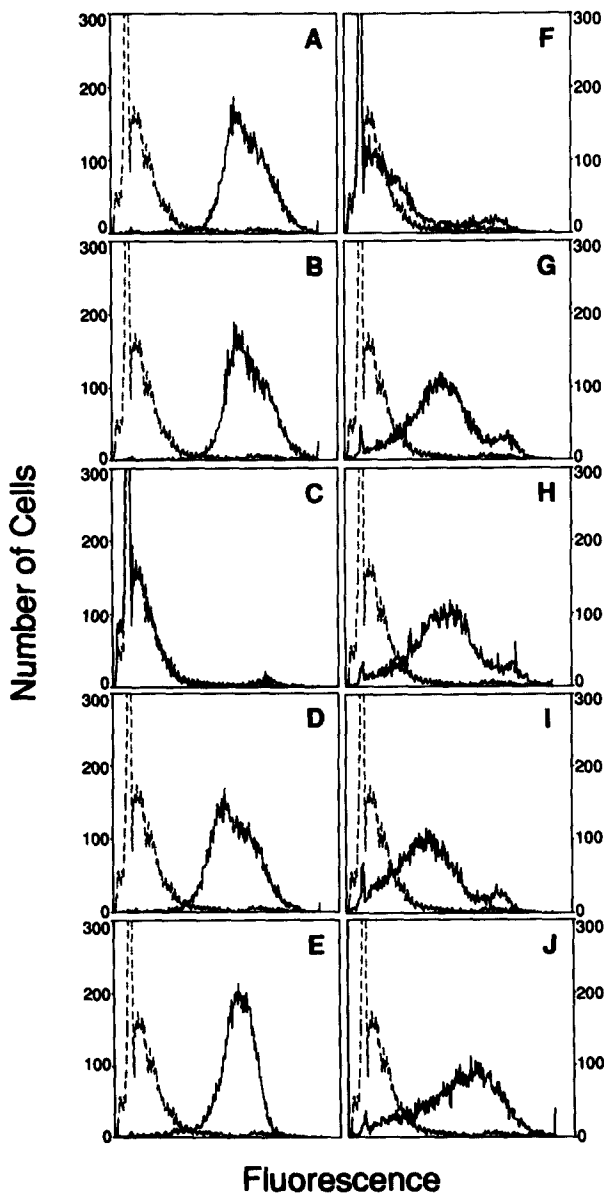


FIGURE 3. Flow cytometric comparison of L cells expressing wild-type CR2 with L cells expressing the chimeric receptor CR2/CR1 XE. L cells stably expressing with pMT:CR2.neo.1 (A-E) or piCR2/CR1 XE (F-J) were indirectly fluorescently labeled with HB-5 anti-CR2 (A and F), OKB-7 anti-CR2 (B and G), YZ-1 anti-CR1 (C and H), and EBV (E and J), respectively, and directly labeled with FITC-pC3dg (D and I), and analyzed by flow cytometry. Nonspecific fluorescent labeling with UPC-10 is shown by the dotted lines.

to untransfected L cells (data not shown). L cells expressing the CR2/CR1 EX chimera bound OKB-7, YZ-1, pC3dg, and EBV but not HB-5 (Fig. 3). The fluorescence intensities of the CR2/CR1 EX and wild-type transfectants binding C3dg and EBV, respectively, were comparable to the fluorescence of these two cell types binding OKB-7, YZ-1 or HB-5, suggesting that the chimeric receptor was equivalent to wild-type CR2 in its capacity to bind EBV and pC3dg.

Quantitative binding of ^{125}I -labeled pC3dg and gp350/220 was performed on these L cell lines to determine whether the chimeric receptor was capable of binding each ligand with the same affinity as that of the wild-type receptor. Replicate samples of wild-type CR2 transfectants, CR2/CR1 XE transfectants, untransfected L cells, and Raji B-lymphoblastoid cells, were incubated with $0.1 \mu\text{g/ml}$ ^{125}I -pC3dg in the absence or presence of incremental concentrations of unlabeled pC3dg, or with $0.25 \mu\text{g/ml}$ ^{125}I -labeled gp350/220 in the absence or presence of incremental concentrations of unlabeled gp350/220, after which bound and free ligand were separated by sedimentation of cells through dibutyl/dinonyl phthalate. Specific competition of the uptake of ^{125}I -labeled ligand with unlabeled ligand was observed with both pC3dg and gp350/220 by L cells expressing wild-type and chimeric receptors as well as by Raji cells, but not by untransfected L cells (Fig. 4). In addition, preincubation

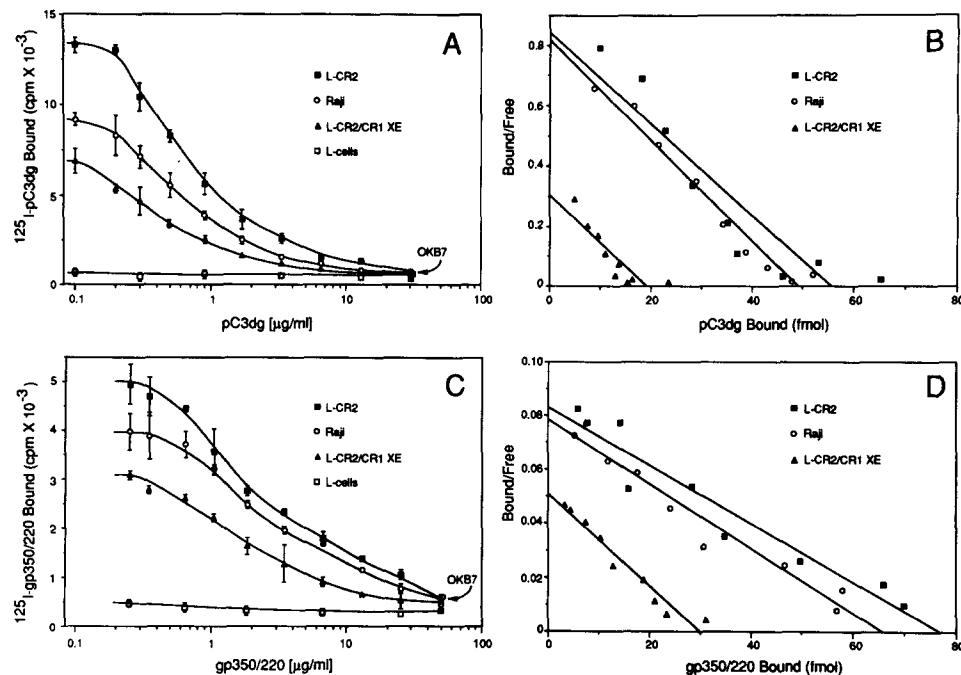


FIGURE 4. Binding of ^{125}I -pC3dg and ^{125}I -gp350/220 to L cells expressing wild-type CR2 or CR2/CR1 XE and to Raji cells. Replicate samples of L cells stably expressing pMT.neo.CR2 or piCR2/CR1 XE, wild-type L cells, and Raji cells and were incubated with ^{125}I -pC3dg (A) or ^{125}I -gp350/220 (C) alone and in the presence of increasing amounts of the corresponding ligands, after which cell-bound ^{125}I -labeled ligand was determined. Binding of each ligand to cells preincubated with OKB-7 is indicated by the arrows. Scatchard analyses of the binding of ^{125}I -pC3dg (B) and ^{125}I -gp350/220 (D) are shown. Lines represent the least squares fit of all data points that are the means of triplicate (^{125}I -pC3dg) or duplicate (^{125}I -gp350/220) determinations.

of each cell type with OKB-7 abolished specific binding of both ^{125}I -pC3dg and ^{125}I -gp350/220, confirming the binding of both ligands to CR2. Scatchard analysis of the binding data indicated comparable affinities for each ligand on all three types: $K_d = 0.7, 0.6,$ and 0.7 nM for pC3dg, and $9.3, 5.9,$ and 8.4 nM for gp350/220 on L CR2, L CR2/CR1 XE, and Raji cells, respectively (Fig. 4). The predicted number of CR2 receptors per Raji cell, which was 3×10^4 with pC3dg and 4×10^4 with gp350/220 and the affinity of CR2 for gp350/220 are in accord with the results of previous studies (44). In summary, these experiments demonstrate that the CR2/CR1 XE chimera is capable of binding both pC3dg and gp350/220 with the same affinity as that of wild-type receptor expressed on L cells or naturally present on Raji cells.

Discussion

Viral tropism is determined in part by the tissue-specific expression of membrane receptors. The capacity of viruses to maintain this functional interaction with target cells requires conservation of both viral envelope protein and receptor binding sites. One strategy for preservation of viral residues that contact receptors is the recessed "canyon" present in rhinovirus capsid protomers that is inaccessible to antibody and thereby is protected from selective pressure of the host immune system (45). Similarly, it has been suggested that several viruses have evolved to recognize receptor domains that are resistant to mutation in that they are binding sites for natural ligand. Indirect evidence suggesting that viruses bind to receptor sites proximate or identical to those for natural ligands includes findings that mAbs that block binding of HIV to CD4, rhinovirus to ICAM-1, and EBV to CR2, also inhibit interaction of MHC class II antigens, LFA-1, and C3dg with their respective receptors (27-30). Direct evidence for overlapping but distinct viral and natural ligand binding sites has recently been obtained by analysis of CD4 substitution mutants affecting binding of both HIV-1 gp120 and MHC class II antigens (31, 46). Furthermore, a nine amino acid sequence near the NH_2 terminus of gp350/220 that is homologous to a sequence in C3dg has been shown recently to mediate binding of EBV to CR2, suggesting that these ligands may bind to a common domain on the receptor (26).

To map directly the EBV/C3dg binding site(s) on CR2 a strategy of deletional mutagenesis was considered suitable because CR2 consists of 15 distinct, tandemly aligned structural units, the SCRs, which, by analogy to other members of the SCR superfamily, form an extended, semiflexible filament (19, 20, 47). Thus, each SCR is likely to interact only with adjacent SCRs, and removal of SCRs not involved in formation of the binding sites should not alter ligand binding function, as has been observed with deletion mutants of CR1 (24). The construction and expression of seven CR2 deletion mutants confirmed the utility of this approach and localized the single epitopes recognized by the mAbs HB-5 and B2 to a region including SCR-3, -4, and a portion of SCR-5, and a region including the COOH-terminal portion of SCR-11 through the NH_2 -terminal portion of SCR-14, respectively. Therefore the internal three-quarters of the receptor extending from SCR-3 to SCR-14 are not required for binding of pC3dg, EBV, or OKB-7 (Fig. 1).

In a second approach analogous to a strategy termed homolog-scanning mutagenesis (48), a set of CR2/CR1 chimeras were constructed in which segments of sequences that are derived from a homologous protein, CR1, are substituted for sequences within the functional protein. The rationale for these constructs is that ge-

netically related proteins have similar three-dimensional structures, despite large sequence divergence, so that chimeric recombinant receptors are expected to be stably expressed on the cell surface. In addition, an analysis not only of chimeras containing intact SCRs from CR2 fused to CR1 but also of intra-SCR substitutions was possible. Hence, transferring portions or all of SCR-1 of CR2 to CR1 did not reconstitute CR2 binding function, but a chimera containing both SCR-1 and SCR-2 of CR2 fused to CR1 was necessary and sufficient to restore EBV, pC3dg and OKB-7 binding (Figs. 1, 3, and 4). Moreover, substitution of a portion of SCR-1 of CR2 with sequences from the homologous portion of CR1 in the intra-SCR-1 chimera, CR2/CR1/CR2 KON maintained the OKB-7 epitope but not the binding site for the viral and natural ligands, indicating that binding site for EBV is more closely related to that for C3dg than for OKB-7. When the binding affinities of the CR2/CR1 XE chimera for EBV and pC3dg were compared with those of wild-type CR2 or recombinant full-length CR2, no difference could be discerned, indicating that SCR-3 through SCR-15 do not contribute to the binding of ligand by this receptor (Fig. 4). Thus, a separation between the EBV and the pC3dg binding sites at the level of SCR or sub-SCR domains was not obtained, supporting the concept that the virus has adapted to recognize the binding site for natural ligand.

Localization of the EBV/C3dg binding site to a pair of contiguous SCRs is reminiscent of findings with the α subunit of the IL-2R and CR1. Mutational analysis of the former demonstrated that both of its SCRs were required for binding of IL-2 (49), and deletional mutagenesis of CR1 predicted that its single binding site for C4b and two sites for C3b were each comprised of two SCRs (24). The capacity of two contiguous SCRs to create a novel conformation is also supported by the analysis of the epitope for OKB-7 that requires both SCR-1 and SCR-2 (Fig. 1). Whether the ligand binding sites of other members of the SCR superfamily will be formed by adjacent SCRs is not known, although all of the ~ 20 members of this family contain at least two SCRs (21).

The mapping of the epitopes for OKB-7 and HB-5 to adjacent pairs of SCRs (Fig. 1) contrasts with the marked differences in their biologic properties. For example, pC3dg, UV-inactivated EBV, and OKB-7, but not HB-5, synergize with phorbol ester to induce proliferation of tonsillar B cells, suggesting that binding of ligand to a site in SCR-1 and SCR-2, but not in SCR-3 and SCR-4, transduces a growth signal (12). Although the mechanism for this mode of signal transduction by CR2 is not known, it may involve interaction with other cellular proteins, as has been suggested by the finding of co-capping of the receptor with membrane Ig (9, 50). An example of selective effects of epitope ligation on signal transduction and protein-protein interaction is the finding that mAbs binding to different epitopes on the TCR that differ by as much as 100-fold in their cellular activating properties correspondingly differ in their capacity to induce association of the TCR with CD4 (51). Thus, the selection by EBV, a polyclonal activator of B cells, of a site in CR2 that is proximate or identical to the natural ligand binding site may be related not only to the relative immutability of that site but also to its signal transducing properties.

Summary

Complement receptor type 2 (CR2;CD21), a member of the superfamily of proteins containing short consensus repeats (SCRs), is the B cell receptor for both the

gp350/220 envelope protein of Epstein-Barr virus (EBV), and for the C3dg protein of complement. By analysis of CR2 deletion mutants and chimeras formed with CR1 (CD35) we determined that of the 15 SCRs in CR2, the NH₂-terminal two SCRs are necessary and sufficient to bind both gp350/220 and C3dg with affinities equivalent to those of the wild-type receptor. The epitope for OKB-7, a mAb that blocks binding of both EBV and C3dg and shares with these ligands B cell-activating capabilities, also requires both SCR-1 and SCR-2, whereas mAbs lacking these functions bind to other SCRs. Thus, EBV, a polyclonal activator of B cells, has selected a site that is proximate or identical to the natural ligand binding site in CR2, perhaps reflecting the relative immutability of that site as well as its signal transducing function.

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References

1. De-The', G. 1985. *In* The Herpesviruses. B. Roizman, editor. Plenum Publishing Corp., New York. 25-103.
2. Harada, S., K. Sakamoto, J. K. Seeley, T. Lindsten, T. Bechtold, J. Yetz, G. Rogers, G. Pearson, and D. T. Purtilo. 1982. Immune deficiency in the X-linked lymphoproliferative syndrome. *J. Immunol.* 129:2532.
3. Raab-Traub, N., K. Flynn, G. Pearson, A. Huang, P. Levine, A. Lanier, and J. Pagano. 1987. The differentiated form of nasopharyngeal carcinoma contains Epstein-Barr virus DNA. *Int. J. Cancer.* 39:25.
4. Montagnier, L., J. Gruest, S. Chamaret, C. Dauguet, C. Axler, D. Guetard, M. T. Nugeyre, R. Barré-Sinoussi, J. C. Chermann, J. B. Brunet, D. Klatzmann, and J. C. Gluckman. 1984. Adaptation of lymphadenopathy associated virus (LAV) to replication in EBV-transformed B lymphoblastoid cell lines. *Science (Wash. DC).* 225:63.
5. Weiss, L. M., L. A. Movahed, R. A. Warnke, and J. Sklar. 1989. Detection of Epstein-Barr viral genomes in Reed-Sternberg cells of Hodgkin's disease. *N. Engl. J. Med.* 320:502.
6. Sugden, B. 1989. An intricate route to immortality. *Cell.* 57:5.
7. Fingerroth, J. D., J. J. Weis, T. F. Tedder, J. L. Strominger, P. A. Biro, and D. T. Fearon. 1984. Epstein-Barr virus receptor of human B lymphocytes is the C3d receptor CR2. *Proc. Natl. Acad. Sci. USA.* 81:4510.
8. Nemerow, G. R., C. Mold, V. K. Schwend, V. Tollefson, and N. R. Cooper. 1987. Identification of gp350 as the viral glycoprotein mediating attachment of Epstein-Barr virus (EBV) to the EBV/C3d receptor of B cells: sequence homology of gp350 and C3 complement fragment C3d. *J. Virol.* 61:1416.
9. Tanner, J., J. Weis, D. Fearon, Y. Whang, and E. Kieff. 1987. Epstein-Barr virus gp350/220 binding to the B lymphocyte C3d receptor mediates adsorption, capping, and endocytosis. *Cell.* 50:203.
10. Ahearn, J. M., S. D. Hayward, J. C. Hickey, and D. T. Fearon. 1988. Epstein-Barr virus (EBV) infection of murine L cells expressing recombinant human EBV/C3d receptor. *Proc. Natl. Acad. Sci. USA.* 85:9307.
11. Changelian, P. S., and D. T. Fearon. 1986. Tissue-specific phosphorylation of complement receptors CR1 and CR2. *J. Exp. Med.* 163:101.
12. Bohnsack, J. F., and N. R. Cooper. 1988. CR2 ligands modulate human B cell activation. *J. Immunol.* 141:2569.

13. Carter, R. H., and D. T. Fearon. 1989. Polymeric C3dg primes human B lymphocytes for proliferation induced by anti-IgM. *J. Immunol.* 143:1755.
14. Carter, R. H., M. O. Spycher, Y. C. Ng, R. Hoffman, and D. T. Fearon. 1988. Synergistic interaction between complement receptor type 2 and membrane IgM on B lymphocytes. *J. Immunol.* 141:457.
15. Moore, M. D., N. R. Cooper, B. F. Tack, and G. R. Nemerow. 1987. Molecular cloning of the cDNA encoding the Epstein-Barr virus/C3d receptor (complement receptor type 2) of human B lymphocytes. *Proc. Natl. Acad. Sci. USA.* 84:9194.
16. Weis, J. J., L. E. Toothaker, J. A. Smith, J. H. Weis, and D. T. Fearon. 1988. Structure of the human B lymphocyte receptor for C3d and the Epstein-Barr virus and relatedness to other members of the family of C3/C4 binding proteins. *J. Exp. Med.* 167:1047.
17. Janatova, J., K. B. M. Reid, and A. C. Willis. 1989. Disulfide bonds are localized within the short consensus repeat units of complement regulatory proteins: C4b-binding protein. *Biochemistry.* 28:4754.
18. Lozier, J., N. Takahashi, and F. W. Putnam. 1984. Complete amino acid sequence of human plasma beta 2-glycoprotein I. *Proc. Natl. Acad. Sci. USA.* 81:3640.
19. Bartow, T., L. Klickstein, W. Wong, K. Roux, and D. T. Fearon. 1989. Analysis of the tertiary structure of CR1 by electron microscopy. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 3:A501.
20. Dahlback, B., C. A. Smith, and H. J. Muller-Eberhard. 1983. Visualization of human C4b-binding protein and its complexes with vitamin K-dependent protein S and complement protein C4b. *Proc. Natl. Acad. Sci. USA.* 80:3461.
21. Ahearn, J. M., and D. T. Fearon. 1989. Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21). *Adv. Immunol.* 46:183.
22. Bevilacqua, M. P., S. Stengelin, M. A. J. Gimbrone, and B. Seed. 1989. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science (Wash. DC).* 243:1160.
23. Siegelman, M. H., M. van de Rijn, and I. L. Weissman. 1989. Mouse lymph node homing receptor cDNA clone encodes a glycoprotein revealing tandem interaction domains. *Science (Wash. DC).* 243:1165.
24. 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.
25. Robb, R. J., C. M. Rusk, and M. P. Neepser. 1988. Structure-function relationships for the interleukin 2 receptor: location of ligand and antibody binding sites on the Tac receptor chain by mutational analysis. *Proc. Natl. Acad. Sci. USA.* 85:5654.
26. Nemerow, G. R., R. A. Houghten, M. D. Moore, and N. R. Cooper. 1989. Identification of an epitope in the major envelope protein of Epstein-Barr virus that mediates viral binding to the B lymphocyte EBV receptor (CR2). *Cell.* 56:369.
27. Rao, P. E., S. D. Wright, E. F. Westberg, and G. Goldstein. 1985. OKB7, a monoclonal antibody that reacts at or near the C3d binding site of human CR2. *Cell. Immunol.* 93:549.
28. Doyle, C., and J. L. Strominger. 1987. Interaction between CD4 and class II MHC molecules mediates cell adhesion. *Nature (Lond.).* 330:256.
29. Peterson, A., and B. Seed. 1988. Genetic analysis of monoclonal antibody and HIV binding sites on human lymphocyte antigen CD4. *Cell.* 54:65.
30. Staunton, D. E., V. J. Merluzzi, R. Rothlein, R. Barton, S. D. Marlin, and T. A. Springer. 1989. A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell.* 56:849.
31. Clayton, L. K., M. Sieh, D. A. Pious, and E. L. Reinherz. 1989. Identification of human CD4 residues affecting class II MHC versus HIV-1 gp120 binding. *Nature (Lond.).* 339:548.
32. White, J. M., and D. R. Littman. 1989. Viral receptors of the immunoglobulin superfamily. *Cell.* 56:725.
33. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain terminating

- inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463.
34. Toneguzzo, F., S. Glynn, E. Levi, S. Mjolsness, and A. Hayday. 1988. Use of a chemically modified T7 DNA polymerase for manual and automated sequencing of supercoiled DNA. *Biotechniques.* 6:460.
 35. Seed, B. 1987. An LFA-3 cDNA encodes a phospholipid-linked membrane protein homologous to its receptor CD2. *Nature (Lond.)* 329:840.
 36. Weis, J. J., T. F. Tedder, and D. T. Fearon. 1984. Identification of a 145,000 Mr membrane protein as the C3d receptor (CR2) of human B lymphocytes. *Proc. Natl. Acad. Sci. USA.* 81:881.
 37. Hoffman, G., S. G. Lazarowitz, and S. D. Hayward. 1980. Monoclonal antibody against a 250,000-dalton glycoprotein of Epstein-Barr virus identifies a membrane antigen and a neutralizing antigen. *Proc. Natl. Acad. Sci. USA.* 77:2979.
 38. Whang, Y., M. Silberklang, A. Morgan, S. Munshi, A. B. Lenny, R. W. Ellis, and E. Kieff. 1987. Expression of the Epstein-Barr virus gp350/220 gene in rodent and primate cells. *J. Virol.* 61:1796.
 39. Dolyniuk, M., R. Pritchett, and E. Kieff. 1976. Proteins of Epstein-Barr virus I. Analysis of the polypeptides of purified enveloped Epstein-Barr virus. *J. Virol.* 17:935.
 40. Selden, R. F. 1988. Transfection using DEAE-dextran. In *Current Protocols in Molecular Biology*. F. R. Ausubel, R. Brent, R. E. Kingston, et al., editors. Green Publishing Assoc. and Wiley-Interscience, New York. 9.2.1-9.2.2.
 41. Chen, C., and H. Okayama. 1987. High-efficiency transformation of mammalian cells by plasmid DNA. *Mol. Cell. Biol.* 7:2745.
 42. Alberti, S., D. R. Parks, and L. A. Herzenberg. 1987. A single laser method for subtraction of cell autofluorescence in flow cytometry. *Cytometry.* 8:114.
 43. Vik, D. P., and D. T. Fearon. 1987. Cellular distribution of complement receptor type 4 (CR4): expression on human platelets. *J. Immunol.* 138:254.
 44. Tanner, J., Y. Whang, J. Sample, A. Sears, and E. Kieff. 1988. Soluble gp350/220 and deletion mutant glycoproteins block Epstein-Barr virus adsorption to lymphocytes. *J. Virol.* 62:4452.
 45. Rossmann, M. G., E. Arnold, J. W. Erickson, E. A. Frankenberger, J. P. Griffith, H. J. Hecht, J. E. Johnson, G. Kamer, M. Luo, A. G. Mosser, R. R. Rueckert, B. Sherry, and G. Vriend. 1985. Structure of a human common cold virus and functional relationship to other picornaviruses. *Nature (Lond.)* 317:145.
 46. Lamarre, D., A. Ashkenazi, S. Fleury, D. H. Smith, R. P. Sekaly, and D. J. Capon. 1989. The MHC-binding and gp120-binding functions of CD4 are separable. *Science (Wash. DC).* 245:743.
 47. Perkins, S. J., L. P. Chung, and K. B. Reid. 1986. Unusual ultrastructure of complement-component-C4b-binding protein of human complement by synchrotron x-ray scattering and hydrodynamic analysis. *Biochem. J.* 233:799.
 48. Cunningham, B. C., P. Jhurani, P. Ng, and J. A. Wells. 1989. Receptor and antibody epitopes in human growth hormone identified by homolog-scanning mutagenesis. *Science (Wash. DC).* 243:1330.
 49. Neeper, M. P., L. Kuo, M. C. Kiefer, and R. J. Robb. 1987. Structure-function relationships for the IL2 receptor system III. Tac protein missing amino acids 102 to 173 (exon 4) is unable to bind IL 2: detection of spliced protein after L cell transfection. *J. Immunol.* 138:3532.
 50. Tsokos, G. C., G. Thyphronitis, R. M. Jack, and F. D. Finkelman. 1988. Ligand-loaded but not free complement receptors for C3b/C4b and C3d co-cap with cross-linked B cell surface IgM and IgD. *J. Immunol.* 141:1261.
 51. Rojo, J. M., K. Saizawa, and C. A. Janeway. 1989. Physical association of CD4 and the T-cell receptor can be induced by anti-T-cell receptor antibodies. *Proc. Natl. Acad. Sci. USA.* 86:3311.