Defining the CD59-C9 Binding Interaction*

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CD59 is a membrane glycoprotein that regulates formation of the cytolytic membrane attack complex (MAC or C5b-9) on host cell membranes. It functions by binding to C8 (α chain) and C9 after their structural rearrangement during MAC assembly. Previous studies indicated that the CD59 binding site in C9 was located within a 25-residue disulfide-bonded loop, and in C8α was located within a 51-residue sequence that overlaps the CD59 binding region of C9. By peptide screens and the use of peptides in binding assays, functional assays, and computer modeling and docking studies, we have identified a 6-residue sequence of human C9, spanning residues 365–371, as the primary CD59 recognition domain involved in CD59-mediated regulation of MAC formation. The data also indicate that both C8α and C9 bind to a similar or overlapping site on CD59. Furthermore, data from CD59-peptide docking models are consistent with the C9 binding site on CD59 located at a hydrophobic pocket, putatively identified previously by CD59 mutational and modeling studies.

Complement activation leads ultimately to the generation and membrane insertion of a cytolytic protein assembly termed the membrane attack complex (MAC). The MAC is formed by the self-assembly of complement proteins C5b, C6, C7, C8, and from 1 to 18 molecules of C9. Host cells are protected from MAC-mediated lysis by CD59, an 18–21-kDa glycophosphatidylinositol-linked membrane glycoprotein. The MAC appears to play an important role in causing tissue injury following inappropriate complement activation in various ischemic and inflammatory conditions (reviewed in Refs. 1–5), and soluble forms of CD59 have been shown to be therapeutic in rodent models of disease (6, 7). Furthermore, CD59 is sometimes overexpressed on tumor cells, and its expression has been linked to promoting tumor growth and the protection of tumor cells from mAb therapy (8–10). It is therefore of interest to understand the molecular interaction between CD59 and its complement ligands for the goal of engineering effective soluble CD59-based therapeutics for treating inflammatory conditions, or for designing CD59 inhibitory molecules to enhance tumor cell susceptibility to complement.

CD59 functions by binding to the α-subunit of C8 in the C5b-8 complex and preventing subsequent binding of C9, and/or by binding to C9 in the C5b-9 complex and preventing recruitment of additional C9 molecules (11–13). CD59 can only bind to C8α and C9 in the nascent complex after conformation rearrangements of the two proteins that occur during MAC assembly. The activity of CD59 is species selective, and previous characterization of chimeric human/rabbit C8α- and C9-identified regions within the primary sequence of these proteins that interact with human CD59 (14, 15). There is considerable sequence homology between C8α and C9, and the identified CD59 binding sequence in the two proteins aligned to a region just C-terminal to the proposed membrane binding domain that encompassed residues 320–415 in human C8α, and 334–415 in human C9. Peptides derived from within these sequences mapped the primary CD59 binding site to residues 359–384 in C9, a sequence contained between a Cys359-Cys405 disulfide loop in the native protein (16), and to residues 364–415 in C8α. In contrast to the C9 sequence, formation of the corresponding interchain disulfide between Cys975-Cys984 in C8α is required for CD59 binding (14). In addition to these studies, earlier investigations in our laboratory using chimeric human/horse C9 molecules and a peptide derived from the putative hinge region of human C9-identified residues 222–271 as involved in CD59 binding (17).

To better define the region of C9 that interacts with CD59 and to identify CD59-inhibitory peptides, peptides spanning the above identified regions of C9 were prepared and screened for CD59-binding and functional activity. A peptide phage display technique was also employed. A short consensus sequence was identified and allowed modeling of peptide-CD59 docking.

EXPERIMENTAL PROCEDURES

Peptides—Linear peptides were synthesized by GenScript (Piscataway, NJ). Cyclized peptide, cp1, corresponding to sequence 359–384 of human C9 was synthesized by AnaSpec Inc. (San Jose, CA). The cp1 peptide was cyclized by a disulfide bridge formed between the two cysteines at each end of the peptide.

Production of Recombinant Soluble CD59—Recombinant soluble CD59 lacking N-linked glycosylation because of a N18Q mutation was used in all binding and functional assays. N-linked glycosylation is not required for CD59 activity and the N18Q CD59 mutant protein has been previously characterized (18). Removal of this glycosylation site facilitated Pichia expression and protein purification. The coding sequence of human
CD59/N18Q was generated from a previously constructed vector (18) by PCR and cloned into the Pichia pastoris expression vector pPICZαA (Invitrogen). The expression plasmid was transformed into P. pastoris-competent SMD1168H via electroporation, and transformed cells were plated on YPD plates with different concentrations of selecting antibiotic Zeocin (Invitrogen). After determination of the phenotype, methanol utilization plus (Mut^+), or methanol utilization slow (Mut^-) pre-selected clones were subjected to a small scale expression test to select a high expressing clone. The level of soluble CD59 expression was determined by standard Western blot or by Sandwich ELISA using monoclonal and polyclonal antibodies to human CD59. Soluble CD59 was expressed via fermentation in a 5 liter BioFlo 110 Fermentor (New Brunswick Scientific, Edison, NJ) according to a modified protocol based on the Pichia Fermentation Process Guidelines of Invitrogen. After 1-day growth on basal medium, a 12-h glycerol feeding batch, and a 3–4-day methanol feeding batch, fermented culture was harvested and centrifuged. The supernatant was filtered with a 0.22-µm AcroPak 200 filtration filter ( Pall Europe Ltd., Portsmouth, UK), concentrated and dialyzed with 50 mM Tris-HCl, pH 8.0 with a Prep/ScaleTM-TFF1ft² cartridge (Millipore Corporation, Bradford, MA). Soluble CD59 in the concentrated supernatant was then purified by anti-CD59 YTH53.1 antibody affinity chromatography and subsequent Mono Q 5/5 ion exchange FPLC (Amersham Biosciences). Purified protein was checked for purity and folding by 18% SDS-PAGE and Western blotting using a panel of anti-CD59 monoclonal antibodies that recognize conformational epitopes described previously (19).

**Labeling of CD59**—Soluble CD59 was biotin-labeled using a biotin labeling kit (Roche Applied Science). CD59 at 1 mg/ml in PBS was mixed with biotin-7-NHS at a molar reaction ratio of 1:5 (CD59:biotin). Following incubation at room temperature for 2 h with gentle stirring, non-reacted biotin-7-NHS was separated on a Sephadex G-25 column.

**CD59 Binding Assays**—In direct binding assays, CD59 binding to immobilized C9, C8, and cyclic peptide, cp1, was determined. The proteins or peptide were diluted with ELISA coating buffer (0.5 M Na+2CO3 buffer, pH 9.6) to a final concentration of 10 µg/ml and boiled in a water bath for 6 min. The boiled samples were then coated on ELISA plates (200 µl/per well) overnight at 4 °C. Boiled BSA at 1 mg/ml served as control. Wells were then blocked with 20 mg/ml BSA in PBS (2 h at 37 °C), and the plates washed five times with PBS containing 0.05% Tween (PBST). Biotinylated CD59 (100 µl of 2 µg/ml) was then added, and following incubation (2 h, 37 °C), wells were washed five times with PBST. A streptavidin-peroxidase conjugate (Roche Applied Science) at 1:5000 dilution was then added, and plates were incubated at room temperature for 1 h with shaking. Following five washes with PBST, CD59 binding was assessed by color development using Sigma FAST o-phenylenediamine dihydrochloride (OPD) tablets. Absorbance was read at 492 nm and data corrected using corresponding BSA controls. In competitive binding assays, the ability of various peptides to inhibit the binding of biotinylated CD59 to immobilized C8, C9, or cp1 was measured. In these assays, different concentrations of peptide were mixed with biotinylated CD59 (2 µg/ml) containing BSA (1 mg/ml) before addition to wells containing immobilized protein or peptide.

**FPLC and Mass Analysis of CD59-p8 Peptide Interaction**—Soluble CD59 (100 µl, 1 mg/ml) was incubated with peptide p8 (100 µl, 2 mg/ml), resulting in a CD59:p8 molar ratio of ~1:20. Incubations were made in 50 mM Tris-HCl, pH 8.0 at room temperature for 30 min. Incubated samples were mixed with 2 µl of matrix in a clean Eppendorf tube and 1 µl of the matrix/sample mixture applied to a clean spot on the MALDI Target. Voyager STR MALDI TOF (Applied Biosystems) was used to acquire data in Linear Mode from 800–10,000 m/z range. Standard 2 from the Sequazyme Peptide mass standards kit (Applied Biosystems) was used for mass calibration of the instrument prior to data acquisition. Two hundred shots per sample were acquired.

**Complement Lysis Assays**—A CHO cell clone-expressing GPI-anchored human CD59 was used in these assays (19). CHO cells (wild type or expressing CD59) at 60–80% confluency were detached with 5 mM EDTA in PBS, washed once and resuspended in Dulbecco’s modified Eagle’s medium (Invitrogen). Cells at 2 × 10^6/ml in Dulbecco’s modified Eagle’s medium were then incubated at room temperature for 30 min with or without peptides at indicated concentrations. CHO cells were sensitized with rabbit anti-CHO membrane serum and dead cells were counted. Wild-type CHO cells served as a negative control for CD59 complement inhibitory function.

**Statistics**—Analysis was performed by Student’s t test, with p < 0.05 considered significant. Error bars shown in figures represent standard deviation.

**Phage Peptide Display**—Three phage peptide display libraries, Ph.D.-7TM, Ph.D.C7C1TM (Cyclic), and Ph.D.-127TM (New England Biolabs, Beverly, MA), were used to screen for human CD59 binding peptides. Three rounds of ELISA-based screening were carried out according to the manufacturer’s instruction. Peptides were synthesized based on derived consensus sequences (GenScript) and tested for CD59 binding as described above.

**Computer Modeling**—A refined structure derived from the CD59 NMR coordinates was used as the target for docking (19). The NMR data for CD59 shows a number of quite flexible amino acid side chains on the surface including Arg55. An in silico flexibility analysis (20) of the refined CD59 structure identified Arg55 as having the most flexible side chain, and by manipulating the side chain conformation of Arg55, it was possible to open a constriction. Compared with the closed NMR average structure, this open conformation extended an adjacent surface groove previously identified as the putative C9 binding site (19). We used both of these conformations for our...
docking analysis. The first conformation was the refined CD59 structure reported by Huang et al. (19) (referred to as the “closed” conformation CD59). The second CD59 conformation (referred to as the “open” conformation of CD59) was produced by selecting a side chain rotamer of Arg55 as noted above. A 6-mer peptide (VSLAFS, the herein identified C9 consensus sequence that binds to CD59) and 8-mer peptide (DVSLAFSE, synthetic peptide shown to bind CD59) were created as objects in ICM (21) and docked to both the closed and the open refined conformations of CD59, defined by the set of residues within 3 Å of the open structure pocket. A second receptor target was a much larger locus of residues within 6 Å of Arg53. This set included the amino acids previously implicated in C9 binding from mutagenesis experiments and that are also located in the vicinity of the computationally predicted binding pocket (18, 19, 22). Ligand and CD59 conformations were docked with the default ICM “thoroughness” variable set to either 2 or to 100 in order to fully explore the convergence of the docking routine.

RESULTS

Binding of C9-derived Peptides to CD59—Previous studies identified two C9-derived peptides that bind CD59; one composed of residues 247–261 within the putative “hinge” region (17) and another composed of residues 359–384 (16). In an attempt to better define the CD59 binding sequence of C9, shorter peptides spanning these residues were synthesized and characterized in CD59 binding assays. To assay for CD59 binding, the ability of peptide to inhibit the binding of soluble CD59 (sCD59) to immobilized denatured C9 was initially measured. Five peptides of 11 residues spanning the 247–261 sequence and differing by one residue were synthesized, but none of the peptides interacted with CD59 in competitive binding assays (data not shown). We also confirmed data from a previous report showing that the full-length 247–261 “hinge” peptide had a direct membrane perturbing effect that interfered with the functional analysis of CD59-inhibition (23). A second series of 7 peptides of 12 residues each and spanning the 360–383 sequence of C9 were synthesized. At the concentrations tested in this screen, three of the peptides (p1–p3) inhibited the binding of sCD59 to immobilized denatured C9. Five peptides of 11 residues spanning the 247–261 sequence and differing by one residue were synthesized, but none of the peptides interacted with CD59 in competitive binding assays (data not shown). We also confirmed data from a previous report showing that the full-length 247–261 “hinge” peptide had a direct membrane perturbing effect that interfered with the functional analysis of CD59-inhibition (23). A second series of 7 peptides of 12 residues each and spanning the 360–383 sequence of C9 were synthesized. At the concentrations tested in this screen, three of the peptides (p1–p3) inhibited the binding of sCD59 to immobilized denatured C9 to a similar extent as the full-length and cyclized Cys359-Cys384 peptide, cp1 (Fig. 1). A fourth peptide (p4) also inhibited sCD59-C9 binding, albeit to a lesser extent. Sequence alignment of these 4 peptides identified a 6-residue consensus sequence of VSLAFS (Fig. 1) (residues 366–371 of C9). Therefore, an additional peptide comprising the consensus sequence was synthesized and tested. To maintain aqueous solubility, the peptide p8 (DVSLAFSE) was synthesized to contain the naturally occurring charged N- and C-terminal residues on either side of the consensus sequence. (Fig. 3). Note that on a molar basis, there is an approximate 3:2:1 ratio for p8:p2:cp1 at similar μg/ml concentration.

Direct evidence that the p8 peptide binds to CD59 in solution was provided by co-incubating CD59 and peptide, followed by
separation of the complex by gel filtration. Following incubation of CD59 and p8 at a 1:20 molar ratio, two peaks were separated by Superdex 75 FPLC, and were shown to be composed of CD59+p8 and p8 only by mass spectroscopy (supplemental data).

Effect of C9 Peptides on Binding of CD59 to C8—CD59 inhibits MAC formation by binding to both C8α in C5b-8 and to unfolded C9 in the assembling C5b-9 complex. To investigate whether C8α and C9 bind to the same or an overlapping epitope on CD59, the ability of the C9-derived peptides to inhibit CD59 binding to immobilized C8 was tested. The C9-derived peptides cp1 and p8 (the consensus sequence from cp1 that binds CD59) significantly inhibited the binding of CD59 to C8 in a dose-dependent manner (Fig. 5). The scrambled p2 peptide, Scr1, had no effect on the CD59-C8 interaction. Peptide p8 was, nevertheless, significantly less effective than cp1 at inhibiting CD59 binding to C8.

Functional Activity of C9-derived Peptides—Specificity of the peptide interaction with CD59 was further investigated by measuring the ability of the C9-derived peptides to block CD59 activity. Blocking the interaction between membrane-bound CD59 and the assembling C5b-9 complex in the membrane will increase complement-mediated lysis. CHO cells expressing high levels of human CD59 were sensitized with antibody and incubated in a sublytic concentration of human serum. The inclusion of peptides p8, cp1, or p2 during the incubation resulted in a dose-dependent increase in complement lysis of the CHO cells (Fig. 6). Scrambled peptide Scr1 had no effect on complement-mediated lysis. It has been noted previously that hydrophobic peptides can increase complement-mediated hemolysis (erythrocyte lysis) in the absence of CD59. However, none of the peptides at the highest concentration tested enhanced complement-mediated lysis of CHO cells that did not express human CD59 (not shown).

**Phage Peptide Display**—Three phage peptide display libraries (7 residue cyclic and 7 and 12 residue linear) were panned using immobilized CD59, resulting in the isolation of CD59 binding peptides. The peptides isolated were: a single cyclic peptide of (C)KHHSHRY(C), two linear dodecamers of AWREHHTYPPL and SWWSFHIHPNPP, and several peptides with a consensus sequence WPXHXH. However, there was no sequence consensus corresponding to sequence within C8α or C9, even for the short HXHX motif found in all peptides.

Because of the potential significance of identifying peptides that are effective CD59 inhibitors, peptides identified by phage display were synthesized and investigated in CD59 binding and functional assays. None of the peptides had any significant
CD59 Binding Site of C9

Results from this study indicate a 6 amino acid sequence of human C9, spanning residues 366–371, represents the primary CD59 recognition domain involved in CD59-mediated regula-

Peptide Modeling and Docking to CD59—The identification of a 6-residue consensus sequence within C9 that binds to CD59 allowed computer modeling of the CD59-peptide interaction. Previous studies involving CD59 mutagenesis putatively identified the C8/C9 binding site at the membrane distal surface in the vicinity of a hydrophobic groove (18, 19, 22). In addition, a computer modeling method for identifying potential small molecule binding sites identified a small pocket in close proximity to the binding site predicted by the mutagenesis studies (19). In the current study, an in silico flexibility analysis of the CD59 structure identified Arg^{55} as having a particularly flexible side chain, and by manipulating the side chain conformation of Arg^{55}, it was possible to open a constriction across the pocket previously identified by computer modeling. Conformations of CD59 with the smaller “closed” binding pocket and the extended “open” binding pocket are shown in Fig. 7, A and B, and lacking a rationale to prefer either conformation of CD59, we elected to use both of these conformations in our docking analysis. The binding pockets that have a high computationally determined likelihood to be a locus for peptide binding are shown in blue. The open structure pocket (Fig. 7B) has a volume of 201 Å³, and has about one-third more surface area and about 40% more volume than the closed pocket (Fig. 7A). The region of the putative C9 binding site determined by mutagenesis studies is shown in cyan. The cyan surface shows the location of this binding region defined as residues within 6 Å of Arg^{33} and includes Trp^{40}, which lies at a geometric centroid encompassing all the residues that alter CD59-C9 binding when substituted. Independent docking runs were performed to analyze for both targets. We initially performed docking calculations using the 8-residue p8 peptide with independent docking runs to analyze for both targets (blue and cyan in Fig. 7). Despite the large docking region, the best scoring bound peptides lay within the computationally identified binding groove (blue) with overlap into the binding region identified by mutagenesis (cyan). However, with the ICM thoroughness factor set to 2, repeat docking calculations beginning with different probe start positions did not reproduce the same conformations, indicating that the program had not converged (not shown). We therefore performed the docking calculations using the identified 6-residue consensus sequence within C9 that binds to CD59, but with the ICM thoroughness increased to 100. When docking to the closed conformation of CD59, different starting positions of the probe position failed to produce convergent ligand conformations (Fig. 7A and Table 1). However, when the experiment was run with the open conformation of CD59, the successive dockings, beginning with distinct starting positions, did converge to the same ligand conformations with the nearly identical ICM energy scores (Fig. 7B and Table 1).

**DISCUSSION**

Results from this study indicate a 6 amino acid sequence of human C9, spanning residues 366–371, represents the primary CD59 recognition domain involved in CD59-mediated regula-

**TABLE 1**

| 6-mer (VSLAFS) docked *in silico* to closed and open CD59 structures using the ICM program |
|-------------------------------------|---------------------------------|---------------------------------|
| Closed conformation of CD59         | ICM score | Color in Fig. 7A |
| Probe initial position              | −111.59   | Red               |
| Probe position moved 1              | −113.1    | Purple            |
| Probe position moved 2              | −90.73    | Green             |
| Open conformation of CD59          | ICM score | Color in Fig. 7B  |
| Probe initial position              | −129.27   | Red               |
| Probe position moved 1              | −129.48   | Purple            |
| Probe position moved 2              | −129.17   | Green             |
CD59 Binding Site of C9

Specificity of the identified C9-derived peptide sequence that interacts with CD59 (VSLAFS) was shown experimentally by: (i) direct peptide binding to CD59, in both solid phase and solution, (ii) peptide-mediated inhibition of CD59 binding to C9, and (iii) peptide-mediated blockade of CD59 function. The ability of the C9-derived peptides to inhibit the binding of CD59 to C8 indicated that C8α and C9 interact with an overlapping epitope on CD59. A previous report indicated that the primary CD59 interaction in the C8α is contained between residues 320–415, and likely centered around 334–385 (14). This region of C8α aligns with the same region of C9 previously shown to be recognized by CD59 (14), and a 25-residue peptide derived from within this region of C9 (359–384) was subsequently shown to specifically bind CD59 (16). However, the aligned CD59-interacting C8α and C9 sequences exhibit only limited sequence identity and homology, and whether C8α and C9 bind to a similar CD59 site was unexplored. Here we provide experimental evidence that C8α and C9 interact with an overlapping epitope on CD59. A previous report indicated that the primary CD59 interaction in the C8α is contained between residues 320–415, and likely centered around 334–385 (14). This region of C8α aligns with the same region of C9 previously shown to be recognized by CD59 (14), and a 25-residue peptide derived from within this region of C9 (359–384) was subsequently shown to specifically bind CD59 (16). However, the aligned CD59-interacting C8α and C9 sequences exhibit only limited sequence identity and homology, and whether C8α and C9 bind to a similar CD59 site was unexplored. Here we provide experimental evidence that C8α and C9 bind to an overlapping site on CD59. Interestingly, an alignment of the VSLAFS C9-derived sequence with the 320–415 CD59 binding sequence of C8α retrieved a single sequence of relatively high hydrophobic homology: LGIQYE (350–355). In addition, these 6-residue C8α and C9 sequences correspond to similar positions within the aligned sequences of C8α and C9. Whereas our data suggest that the CD59 binding sites of C8α and C9 are structurally similar, it is of note that previous studies have shown that the CD59 binding site of C8α, but not of C9, is conformationally sensitive (C8α requires disulfide bond formation (14, 23)), suggesting that the CD59 binding sites on the two proteins are structurally dissimilar but comprise distinct motifs. Of further interest, the alignment of human, rabbit, horse, rat, and mouse C9 reveals that the region containing the identified 6-residue CD59 binding sequence corresponds to short sequence of particularly low homology (supplemental data). It is possible that this may relate to the species selectivity of CD59.

The identified hydrophobic C9 sequence that interacts with CD59 is likely buried within the protein core, which would be consistent with the known mechanism of action of CD59 in that CD59 does not bind free plasma C9, but binds to C9 as it undergoes a structural rearrangement upon binding to C5b-8 in the nascent complex. Together with data indicating the hydrophobic nature of the binding site on CD59 for C9, it is therefore very likely that C9-CD59 binding occurs through a predominantly hydrophobic interaction, a supposition further supported by molecular modeling studies. The docking studies performed cannot address the native conformation of the complete C9 protein, nor its potential for unfolding prior to interactions with CD59. However, the studies do illustrate that plausible conformations of the core motifs present in the C9 peptide can interact specifically with CD59. The inability of the ICM program to produce convergent ligand conformations with the closed conformation of CD59 suggests this conformation is unproductive for C9 ligand binding. Conversely, the more open CD59 conformation with a larger target pocketsize can support convergent 6-mer docking, suggesting that the active conformation of Arg55 in CD59 in vivo is more likely to be similar to the open than the closed conformation.Taken together, the data from the modeling studies offer plausible interactions to illustrate the means by which C9-derived peptides might interact with the particular locale on the human CD59 surface that has been implicated in C9 binding experimentally by both CD59 mutagenesis (18, 19, 22) and synthetic C9 peptide interaction effects (current study).

We isolated several peptides by phage display that bound to human CD59 and identified four consensus sequences. However, there was no sequence homology with C8α or C9, none of the peptides interfered with CD59 function, and only one peptide inhibited C9 binding to CD59 (and only moderately). CD59 is a multifunctional protein; it binds to cholesterol-dependent cytolysins intermediolysin (24), cell adhesion molecule CD2 (25), calreticulin (26), and possibly, natural cytotoxicity receptors (27). Nothing is known concerning the binding interface between CD59 and these other ligands, but it is possible that phage display-derived peptides may interfere with one or more of these interactions. Indeed, it is also possible that some of these ligands share an overlapping binding site on CD59 with C8α and C9, and that C9-derived peptides interfere with the binding of these other ligands.

We had previously shown that a synthetic peptide derived from the C9 hinge region (247–261) bound CD59 and enhanced complement lysis of human erythrocytes (which are protected by endogenously expressed CD59) (17). However, it was subsequently reported that CD59 did not specifically bind to fusion proteins containing the hinge sequence, and that the enhanced lytic activity was caused by a direct membrane perturbing effect of the peptide (23). Also, in the current study, we were unable to demonstrate CD59 binding of any shorter peptides derived from within the hinge region. Together with the data reported here on the VSLAFS C9 sequence, it is concluded that the hinge region of C9 is not involved in the inhibitory function of CD59.

CD59 is widely expressed on tumor cells, often at elevated levels, and CD59 blockade enhances antibody and complement-mediated tumor cell lysis. On the other hand, the MAC is thought to play a pathogenic role in various inflammatory conditions, and blocking MAC formation may represent a therapeutic option. Understanding the molecular interaction between CD59 and its complement ligands has implications for the design of therapeutic strategies aimed at blocking CD59 activity or blocking MAC formation. The identification of a 6-residue sequence of human C9 as the primary CD59 recognition domain may enable the development of assays and small molecule therapeutics in the area of binding. Understanding the CD59-C9 interaction may also enable rational design of CD59 mutations to engineer improved CD59-based therapeutics, and results from the present study will permit a rational
approach for further investigation of activity-enhancing mutations.

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