Measles Virus Recognizes Its Receptor, CD46, via Two Distinct Binding Domains within SCR1-2

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Measles virus (MV) enters cells by attachment of the viral hemagglutinin to the major cell surface receptor CD46 (membrane cofactor protein). CD46 is a transmembrane glycoprotein whose ectodomain is largely composed of four conserved modules called short consensus repeats (SCRs). We have previously shown that MV interacts with SCR1 and SCR2 of CD46. (M. Manchester *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92, 2303–2307) Here we report mapping the MV interaction with SCR1 and SCR2 of CD46 using a combination of peptide inhibition and mutagenesis studies. By testing a series of overlapping peptides corresponding to the 126 amino acid SCR1-2 region for inhibition of MV infection, two domains were identified that interacted with MV. One domain was found within SCR1 (amino acids 37–56) and another within SCR2 (amino acids 85–104). These results were confirmed by constructing chimeras with complementary regions from structurally similar, but non-MV-binding, SCRs of decay accelerating factor (DAF; CD55). These results indicate that MV contacts at least two distinct sites within SCR1-2. © 1997 Academic Press

INTRODUCTION

Measles virus infections continue to occur despite the use of an effective live-attenuated vaccine. Thirty years after the introduction of vaccination, measles still remains one of the leading causes of infant death worldwide (Bloom, 1989). No specific antiviral therapies for MV infection currently exist. Vaccine failure is often caused by interference from passively acquired maternal antibody (Gellin and Katz, 1994; Katz and Gellin, 1994). In infants, maternal antibodies that protect children from measles during the first year of life also prevent replication of the attenuated vaccine strain. As maternal antibody wanes, a window of susceptibility occurs where wild-type MV can replicate but attenuated vaccines cannot; most MV infections occur within this interval (Katz and Gellin, 1994).

The initial interaction between MV and the host occurs following aerosol transmission with the initial infection in the oral, nasal, and respiratory mucosa (Norrby and Oxman, 1990). Virus subsequently disseminates throughout the body via the lymphoid system, and concomitant immunosuppression can lead to an enhanced morbidity and mortality (Griffin and Bellini, 1996). MV enters cells by attachment of the viral hemagglutinin protein (H) to a cellular receptor, and subsequently direct fusion with the cell membrane mediated by the viral fusion protein (F) (Wild *et al.*, 1991; Nussbaum *et al.*, 1995). The measles virus receptor, CD46 or membrane cofactor protein (MCP), is a transmembrane glycoprotein of MW 57–67 kDa and a member of the Regulators of Complement Activation (RCA) superfamily (Liszewski *et al.*, 1991; Dorig *et al.*, 1993; Naniche *et al.*, 1993; Manchester *et al.*, 1994). RCA proteins protect the host from autologous complement (Liszewski *et al.*, 1991). CD46, in particular, binds the complement activation fragments C3b and C4b deposited on the cell surface and serves as a cofactor for their cleavage by the serine protease factor I (Seya *et al.*, 1986). In this manner further complement deposition on the cell surface is prevented and complement-dependent phagocytosis or lysis of host tissue is prevented.

The structure of CD46 and its regulatory activity have been well characterized. The extracellular domain of CD46 is composed, beginning at the amino terminus, of four short consensus repeat modules (SCRs). SCR modules are found in all members of the RCA superfamily and serve as the complement regulatory sites (Liszewski *et al.*, 1991). Each SCR module is approximately 60 amino acids in length and contains four invariant cysteines forming two disulfide bonds along with a number of other conserved residues. These framework requirements lead to an approximately 30% amino acid similarity among the modules of different RCA family members. A single SCR forms a beta-barrel with a compact core, and a number

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of exposed, less-conserved regions that are thought to be candidates for ligand-binding (Barlow *et al.,* 1991, 1992). For CD46, C3b- and C4b-binding activity maps to SCRs 2, 3, and 4 with cofactor activity particularly contributed to by SCR2 (Adams *et al.,* 1991). In contrast, the MV receptor determinant on CD46 lies in the ectodomain within SCRs 1 and 2, distinct from the complement regulatory domains (Iwata *et al.,* 1995; Manchester *et al.,* 1995; Buchholz *et al.,* 1996). Both SCR1 and SCR2 modules are required for successful MV binding and infection (Iwata *et al.,* 1995; Manchester *et al.,* 1995; Buchholz *et al.,* 1996).

To further characterize the interaction between MV and SCR 1-2, an approach that combined peptide inhibition and mutagenesis was used. These mapping studies defined two regions of CD46 within SCR1-2 that are involved in virus attachment. Further, peptide inhibition experiments provide evidence that small soluble peptides based on the linear sequence of the receptor can inhibit MV infection.

MATERIALS AND METHODS

Virus, cells, and media

Measles virus (MV) Edmonston strain was obtained from the ATCC and low-multiplicity passaged stocks of virus were generated and titered on Vero cells. Virus was purified by sucrose gradient centrifugation as described (Manchester *et al.,* 1995) and frozen in aliquots at $-70°$. Protein determinations were made of each virus stock by the Bradford assay. Vero cells were obtained from the ATCC and maintained in M199 medium supplemented with 7% fetal bovine serum and 2 mM L-glutamine. CHO cells were grown in Ham's F12 medium supplemented with 7% fetal bovine serum, 2 mM L-glutamine, and 0.5 mg/ml Geneticin (G418, Gibco BRL). HeLa cells were maintained in Dulbecco's MEM supplemented with 7% fetal bovine serum and 2 mM L-glutamine.

Synthesis and preparation of peptides

The initial set of 27 CD46 peptides corresponding to SCR1-2 were synthesized by Chiron Mimotopes (La Jolla, CA). Each peptide in this set contained an amino-terminal biotin followed by a four-amino acid (Ser-Gly-Ser-Gly) spacer. In addition we synthesized 12-amino acid peptides on an Applied Biosystems synthesizer without the N-terminal biotin label or spacer. Methods of peptide synthesis and determining peptide purity by HPLC and mass spectrometry have been described (Gairin and Oldstone, 1992; Oldstone *et al.,* 1995). In addition, control 20-mer peptides were used having sequences unrelated to CD46. All peptides were reconstituted in 20% DMSO in PBS at a concentration of 2 mM and stored at $-20°$ prior to use. Peptides were subsequently diluted in PBS to the working concentrations indicated.

Inhibition of MV infection using peptides

Peptides were diluted to 100 or 10 μM in PBS. All assays were performed in duplicate. Peptides were incubated with 2×10^5 PFU of sucrose-gradient-purified MV for 20 min on ice and then incubated with 1×10^5 Hela cells. After 1 hr at $37°$ the cells were washed four times in PBS and then incubated with DMEM containing 7% FBS for 24 hr at $37°$. Soluble CD46 (sCD46; 0.1 μM) was included as a positive control and PBS alone as a negative control for all experiments. Cells were fixed and stained with a polyclonal human antiserum to MV, followed by a FITC-conjugated anti-human secondary antibody. Percentage of MV-infected cells was determined by immunofluorescence microscopy. A minimum of four fields was counted per sample, and all samples were coded so that the quantitation was unbiased. All data are expressed as the mean for each sample $\pm$ S.E. Results were reproducible over four independent experiments.

Hemagglutination assay

The assay was performed according to Norrby and Gollmar (1975) with the following modifications. Peptides were diluted to 10^{-4} or 10^{-5} M in 90 μl DMEM, 7% FBS in individual wells of a round-bottom 96-well plate (Corning, Corning NY). Edmonston MV (0–10 μl/well for controls or 5 μl/well for peptide assays; 1×10^7 PFU/ml) was added to each well and incubated for 10 min at $37°$. After this initial preincubation, 10 μl of a 2% african green monkey red blood cell suspension (BioWhittaker, Walkersville, MD) in DMEM with 7% FBS was added to each well and gently mixed bringing the total volume to 100 μl. The microtiter plate was incubated at $37°$ for 2 hr, allowing the red blood cells to agglutinate and/or settle to the bottom of the wells. Hemagglutination was scored as a uniform distribution of cells over the bottom of the well (+ agglutination) or the presence of a cluster or dark spot of cells at the bottom of the microtiter wells (no agglutination).

Construction of CD55 substitutions into CD46

All mutants were constructed in the plasmid vector pHβApr.1.neo (Gunning *et al.,* 1987). CD46 (BC1 isoform) cDNA was previously described (Oglesby *et al.,* 1991). A PCR mutagenesis strategy was used to introduce CD55 sequences into the CD46 cDNA. Specifically, for the chimera 1S4, the CD55 amino acid sequence LKGSQWSDIEEFCN was substituted into CD46 SCR1 while deleting DRNHTWLPVSDDACY. For the chimera 2A6, the CD55 amino acid sequence FPVGTVVEYECRPGYRREPSLSPK was substituted into CD46 SCR2 while deleting FGYQMHFICNEGYYLIGEEI. To do this, oligonucleotide primers were designed to create two segments for each chimera, 1S4-A and 1S4-B, or 2A6-A and 2A6-B, each possessing *Eco*RI

sites externally and CD55 sequence internally. These were subsequently ligated together to create the CD55 substitutions into CD46. For 1S4, segment A was amplified using as the 5′ primer GGAATTCCTCTGCTTTCCTCCGGAGAAATA (CD46 5′ untranslated) and the internal phosphorylated 3′ primer ATCTGACCATTGACTGCCCTTAA GACAAATAG-TATGGGTGGCAAGAGG (CD46 + CD55). 1S4 segment B was created using the external primer GGAATTCCCAAG-CCACATTGCAATATT AGCTAAGCCACA (CD46 3′ untranslated region) and the phosphorylated internal primer ATT-GAAGAGTTCTGCAATAGAGAAACATGT CCATATATACGG (CD55 + CD46). 2A6 segment A was amplified using the 5′ primer GGAATTCCTCTGCTTTCCTCCGGAGAAATA (CD46 5′ untranslated) and the internal phosphorylated 3′ primer ACGGCACTCATATTCCACAACAGTACCGACTGGAAACT-CGTAAGTCCCATTTGCAGGGACTGCTTGGCC (CD46 + CD55). 2A6 segment B was created using the external primer GGAATTCCCAAGCCACATTGCAATATTAGC-TAAGCCACA (CD46 3′ untranslated region) and the phosphorylated internal primer CCAGGTTACAGAAGAGAACCT-TCTCT ATCACCAAAACTATATTGTGAACTTAAAGGATCA-GTAGCAATTTGGAGC (CD55 + CD46). PCR reactions were performed using PFU polymerase (Stratagene, La Jolla, CA) and reactions were performed as follows. For each PCR cycle denaturing was at 95° for 2 min and extension at 72° for 2 min. The annealing temperatures were increased as follows; the first 4 cycles annealing at 37°, the next 4 cycles annealing at 45°, and the final 30 cycles annealing at 55°. The PCR products A and B for each chimera were gel purified and ligated together. A second round of PCR was performed on the ligation mixture using the CD46 external primers and the same reaction conditions as above. The fragment was gel purified and cloned using the TA cloning kit (InVitrogen) and colonies selected on kanamycin. The entire CD46/CD55 chimera was sequenced to confirm the junctions and then inserted as an EcoRI fragment into the vector pHβapr.1.neo (Gunning et al., 1987). Construction of the NQ1 mutant has been previously described (Maisner et al., 1996). Recombinant clones were introduced into CHO cells by cationic lipid transfection (Lipofectin, Gibco BRL, Bethesda, MD) and colonies selected using 0.5 mg/ml G418 (Geneticin, Gibco BRL, Bethesda, MD). Resistant cells were pooled and sorted for high expression of wild-type or mutant CD46 by flow cytometry. CD46 expressing cells were tested using a panel of monoclonal antibodies against each SCR to confirm that the protein was properly expressed at levels similar to wild-type CD46 (Manchester et al., 1995).

Infection of CHO transfectants

CHO cells expressing various CD46 and CD55 chimeras were infected with purified MV at a m.o.i. of 2. At 24 and 48 hr postinfection, cells were fixed and stained using a polyclonal human antiserum to MV followed by an anti-human FITC-conjugated secondary antibody. Per-

centage of infected cells was quantitated using fluorescence microscopy. A total of four fields per sample was counted and the mean values were indicated. Standard error for all samples was less than 5%. Concurrent with the infection, transfectants were stained with monoclonal antibodies against CD46 and analyzed by flow cytometry to confirm that receptor expression levels were similar for all transfectants (Cho et al., 1991; Manchester et al., 1995).

Immunohistochemical staining of cells infected with MV and cultured in the presence of sCD46 was performed using a Vectastain Elite kit (Vector Labs). Cells on glass coverslips were blocked with avidin and biotin, stained with a human antiserum to MV, followed by a biotinylated secondary antibody and a streptavidin-peroxidase reagent. Recombinant sCD46 was produced in CV-1 cells and was provided by CytoMed, Inc. (Cambridge, MA).

Molecular modeling of SCR1-2 of CD46

An alignment of the amino acid sequences of SCRs15-16 from factor H and SCRs 1-2 of CD46 was performed using Geneworks version 2.2.1 (Intelligenetics, Inc.). The coordinates for factor H SCR15-16 were obtained from the Brookhaven Protein Data Bank (file 1hfh) (Barlow et al., 1993). Model of SCR1-2 based on factor H SCR15-16 was generated using the program O (Jones et al., 1991).

RESULTS

Peptide mapping of the CD46-MV interaction

To determine if MV infection could be blocked by interfering with the MV–receptor interaction, we studied the ability of soluble CD46 (sCD46) truncated at the transmembrane domain or peptides corresponding to SCR1 and SCR2 to block MV infection. For sCD46, serial 10-fold dilutions of sCD46 or BSA were mixed with 2×10^5 PFU of sucrose-gradient purified Edmonston MV. At 24 hr postinfection, cells were stained for MV antigen and the percentage of infected cells quantitated (Fig. 1A). At this multiplicity of infection 95% inhibition was seen at 10 nM with half-maximal inhibition at 0.05 nM. In contrast, the same concentrations of BSA did not inhibit MV infection. These results indicate that CD46 need not be attached to the cell membrane to interact with MV.

A significant portion of virus spread in MV infection occurs via cell–cell transmission (syncytium formation) rather than production of extracellular virions (Norrby and Oxman, 1990). To determine whether sCD46 could inhibit syncytium formation in infected cells, confluent monolayers of HeLa cells were infected at a m.o.i. of 0.1. At 24 hr postinfection sCD46 was added to the cultures at concentrations of 1, 10, and 100 nM and incubated for a further 24 hr. In this assay sCD46 reduced the number of cells involved in syncytia by 10-fold at 100 nM (data
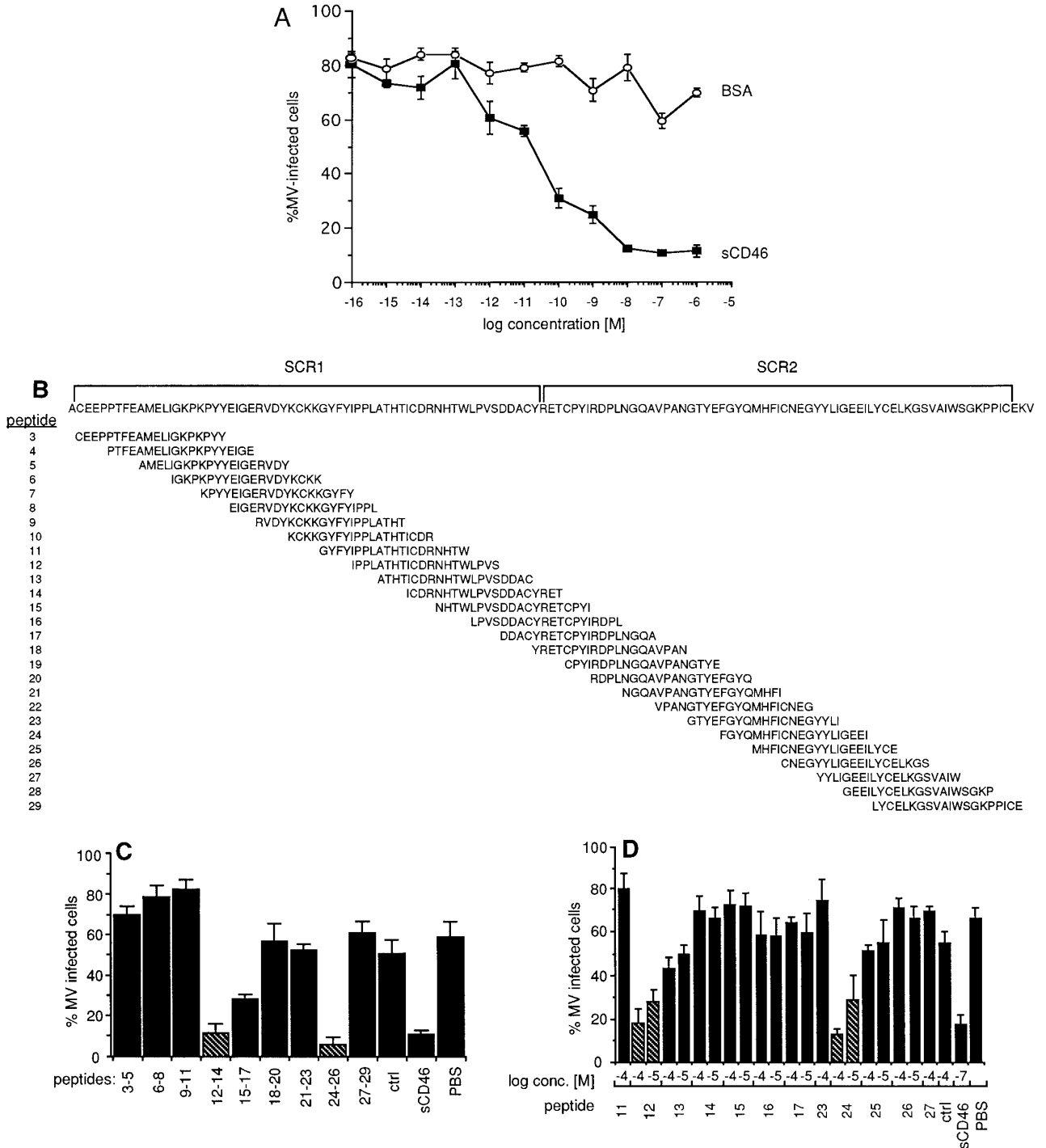
FIG. 1. Inhibition of MV infection by peptides corresponding to SCR1-2 of CD46. (A) Inhibition of MV infection and cell–cell spread by sCD46. sCD46 was diluted in PBS, incubated with MV (2×10^5 PFU) for 10 min on ice and then added to 5×10^4 HeLa cells. After 24 hr, cells were fixed and stained for immunofluorescence as described under Materials and Methods, and percentage of MV-positive cells was determined by fluorescence microscopy. Values indicated are mean of at least 4 fields $\pm$ S.E. (B) Twenty-amino-acid overlapping peptides are depicted in single-letter code. (C) Peptides were tested in nine groups of three for MV-inhibitory activity. Peptides were diluted to 100 μM and then added to MV for 10 min on ice. The peptide–MV mixture was added to HeLa cells for 1 hr and then washed 3 times with PBS. Infected cells were quantitated by fluorescence microscopy at 24 hr postinfection and at least 4 fields were quantitated from duplicate samples. Values are expressed as mean $\pm$ S.E. (D) Peptides 12–17 and 23–27 from C were tested at 100 and 10 μM. Inhibition of MV infection was quantitated at 24 hr postinfection as in C.

not shown), indicating sCD46 can block cell–cell spread among already infected cells.

We have previously shown that the MV receptor de-terminant lies within SCR1 and SCR2 of CD46 (Man-chester *et al.*, 1995). Using a series of 20-amino-acid overlapping peptides, the regions within these two

A

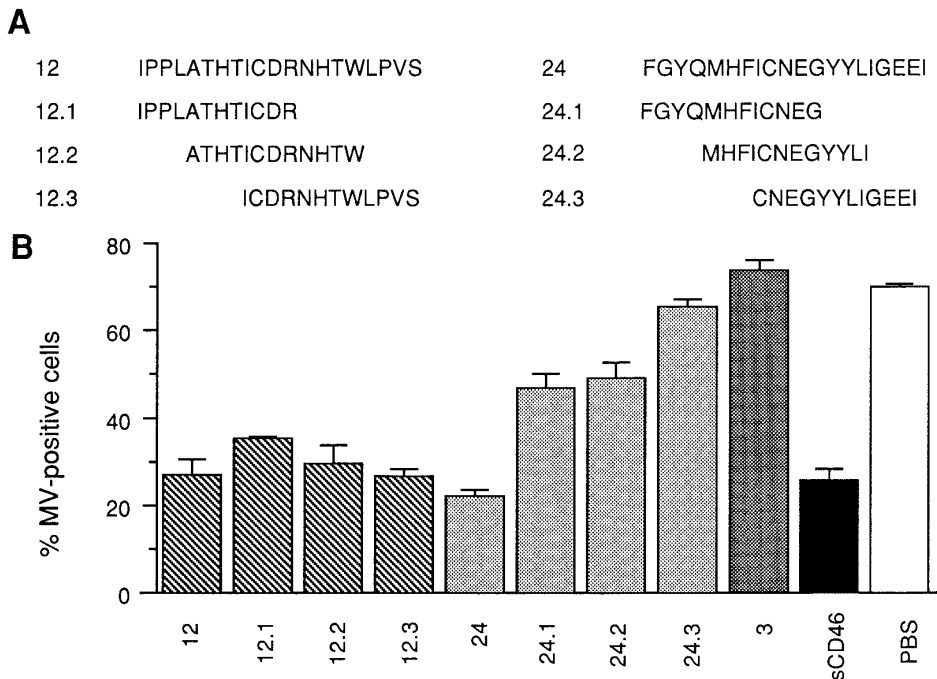| 12	IPPLATHTICDRNHTWLPVS	24	FGYQMHFICNEGYYLIGEEI
12.1	IPPLATHTICDR	24.1	FGYQMHFICNEG
12.2	ATHTICDRNHTW	24.2	MHFICNEGYYLI
12.3	ICDRNHTWLPVS	24.3	CNEGYYLIGEEI

B

FIG. 2. Inhibitory activity of small peptide derivatives of peptides 12 and 24. (A) 12 amino acid peptides were synthesized as shown. (B) Peptides were tested at 100 μM for MV-blocking activity, and values are depicted as means $\pm$ S.E. Peptide 3 (from B) is a negative control, while 0.1 μM sCD46 is a positive control for inhibition.

SCRs required for virus binding were mapped. Peptides were designed that spanned the entire 124-amino-acid SCR1-2 region, overlapping by five amino acids (Fig. 1B). Each peptide was initially tested for its ability to inhibit MV infection at 100 μM. Initially the 27 peptides were tested in groups of 3, using 0.1 μM sCD46 as a positive control for inhibition (Fig. 1C). At the concentration tested, three groups of peptides inhibited MV infection with an efficiency similar to (peptides 12–14 and 24–26) or lower than (peptides 15–17) that of sCD46. The six other groups did not differ significantly from PBS alone or a negative control group composed of three 20-mer peptides unrelated to CD46 (see Materials and Methods). Peptides from the inhibitory groups were then tested separately at 100 and 10 μM (Fig. 1D). In this assay, two peptides showed a dose-dependent inhibition of MV infection: peptides 12 (IPPLATHTICDR-NHTWLPVS) and 24 (FGYQMHFICNEGYYLIGEEI) (Fig. 1D). Peptide 12 corresponds to amino acids 37–56 (numbering from the amino terminus of the mature CD46 protein) in SCR1, spanning the third cysteine. Peptide 24 corresponds to a region spanning the second cysteine in SCR2, amino acids 85–104. Interestingly, while the combination of peptides 15–17 showed slight inhibition (Fig. 1C), none of these peptides showed inhibition on their own. It may be that each of these peptides has slight inhibitory activity and the combination is additive.

Using peptides 12 and 24 as starting points, 12-amino-acid-long peptides with 8 residue overlapping regions were synthesized (Fig. 2A). Within peptide 12

(SCR1 amino acids 37–56), the three 12-amino-acid derivatives (12.1, 12.2, 12.3) showed similar inhibition to peptide 12 (Fig. 2B). The sequence ICDR is common to all these peptides. Peptide 12 also overlaps a site for N-linked glycosylation in SCR1 (asparagine 49). Although the peptides do not contain N-glycans, N-linked glycosylation of the CD46 ectodomain is known to be important for MV binding and infection (Maisner et al., 1994; Maisner and Herrler, 1995) (see below). Peptide secondary structure in this region may also be important for binding to MV H.

Within peptide 24 (SCR2 amino acids 85–104), three smaller peptide derivatives were also synthesized (24.1, 24.2, 24.3). As seen in Fig. 2B, the smaller derivatives containing the N-terminal or central regions (24.1 and 24.2) showed some inhibition of infection, but none of the smaller derivatives were able to inhibit MV infection as well as the full-length peptide 24. These results indicate that the full 20-amino-acid peptide is required for maximal MV binding but that the amino-terminus is more important for recognition. These results are supported by the finding that peptide 25 (Figs. 1C and 1D) also shows slight inhibition at 100 μM. Peptides in this region also may have secondary structure that is important for recognition by MV H.

Titration of the inhibitory effects of peptides 12 and 24 was performed. Peptide 12 showed half-maximal blocking activity at 50 μM, while peptide 24 showed half-maximal blocking activity at 0.1 μM (data not shown). Peptides 12 and 24 were tested in combination to deter-
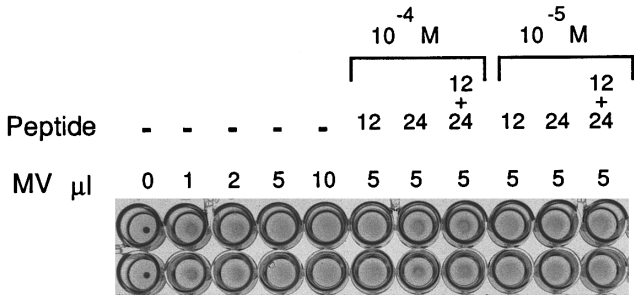
FIG. 3. Inhibition of hemagglutination activity by CD46-mimetic peptide 24. Hemagglutination assay was performed in 96-well round bottom plate in duplicate. In the first five pairs of wells no peptide was added, and increasing amounts of MV (1, 2, 5, and 10 μl, corresponding to 1×10^4, 2×10^4, 5×10^4, and 1×10^5 PFU of MV) was used to agglutinate the red blood cells. In the last six columns peptides 12, 24, and 12 + 24 were added at either 10^{-4} or 10^{-5} M, in combination with 5 μl (5×10^4 PFU) of MV. Hemagglutination was complete after 2 hr at 37°. Whereas agglutinated red blood cells spread in a uniform layer over the bottom of the well, inhibition of hemagglutination by peptide is indicated by a dark circle in the center of the well.

mine if they showed additive or synergistic effects. When the peptides were tested in combination at 100 μM, they showed a 15% increase in inhibition compared to either peptide alone (data not shown). This inhibition was equivalent to the amount of blocking seen using monoclonal or polyclonal antibodies against CD46, where approximately 95% of MV infection can be inhibited (Manchester et al., 1994). The remaining 5% of cells are infected independent of CD46, as CHO cells and other cell types lacking CD46 can be infected by MV at this low level (see Fig. 4 and Dorig et al., 1993; Naniche et al., 1993; Manchester et al., 1994; Manchester et al., 1995).

To confirm the inhibitory activity of peptides in a separate assay, the ability of MV blocking peptides 12 and 24 to inhibit hemagglutination was assessed. In the MV system hemagglutination is defined as the ability for the MV hemagglutinin glycoprotein to interact with CD46 on the surface of African green monkey red blood cells (RBC). MV was mixed with peptides 12 or 24, or the combination, for 20 min and then incubated with 10 μl of a 2% African green monkey red blood cell suspension bringing the final concentration of RBCs to 0.2%. After 2 hr at 37° hemagglutination activity was assessed. In this assay, in the absence of hemagglutination, the cells will collect at the bottom of the well and form a dark spot. When hemagglutination of the RBCs occurs the cells form a matte across the bottom of the well. As seen in Fig. 3, in the absence of peptide increasing amounts of virus added to the wells leads to increasing hemagglutination (columns 1–5). When peptide 24, the most effective MV blocking peptide was added, hemagglutination was decreased (compare wells with 5 μl of MV, plus or minus peptide 24). When peptide 12 was added, little or no decrease in hemagglutination activity was seen. This result was reproducible over four separate experiments,

using different batches of RBCs. Thus in this assay the ability of peptide 24 to inhibit hemagglutination (which measures direct binding of MV H to CD46) is consistent with the results of the infection–inhibition assay. It is not clear why peptide 12 cannot block hemagglutination when it does inhibit infection. It may be that the numbers of CD46–MV H interactions required for achieving hemagglutination may be so high that even a high concentration of the weaker blocking peptide 12 (100 μM) is not enough to block the interaction, while the stronger blocking peptide 24 is able to compete with the CD46-MV H interaction. This is in contrast to the infection–inhibition assay (Fig. 1) where a smaller number of CD46–MV H interactions are probably sufficient to achieve infection and thus peptide 12 can inhibit.

Mutagenic analysis of CD46 MV-binding regions

To confirm that regions of CD46 identified by peptide mapping could indeed interact with MV, mutations in SCR1 and SCR2 were generated and tested for MV receptor function. In this strategy, a small analogous region of SCR1 or SCR2 of the complement regulatory protein decay accelerating factor (DAF; CD55) was substituted for the corresponding regions of CD46. CD46 and CD55 share 40% amino acid similarity within SCR1-2. We have previously shown that CD55 does not bind to MV (Manchester et al., 1995). Mutants were constructed by PCR mutagenesis based on comparing regions of amino acid similarity between SCR1-2 of CD46 and CD55. 1S4 substitutes the region of CD46 SCR1 corresponding to the sequences of peptides 12.3 and 13 (Fig. 4) with sequences from CD55 (see legend). 2A6 substitutes the sequence of CD46 SCR2 corresponding to peptides 24, 24.1, 24.2, and 24.3 with sequences from CD55. Each of these plasmids was introduced into otherwise nonpermissive CHO cells, and a receptor-expressing cell line selected by G418 resistance was sorted for higher expression by FACS. CHO cells expressing wild-type CD46, wild-type CD55 (Manchester et al., 1995), and a mutation of asparagine 49 to glutamine (designated NQ1) (Maisner et al., 1996) were also tested. CHO cells expressing each of the receptors shown in Fig. 4 at similar levels were infected with MV at an m.o.i. of 2 and the percentage of infected cells was determined at 24 hr postinfection. Values are expressed relative to infection of cells expressing wild-type CD46 that is set at 100%. As seen in Fig. 4, the 1S4 mutant showed a 50% decrease in MV infection compared to cells expressing wild-type CD46. These results confirm the peptide inhibition studies using peptides 12, 12.3, and 13 (Figs. 1C and 2) and further establish that this region of SCR1 plays a role in MV recognition. Since the 1S4 mutant overlapped a site of N-linked glycosylation, the mutant that substituted asparagine 49 with glutamine (NQ1) was also tested in this assay. Removal of the oligosaccharide addition signal at
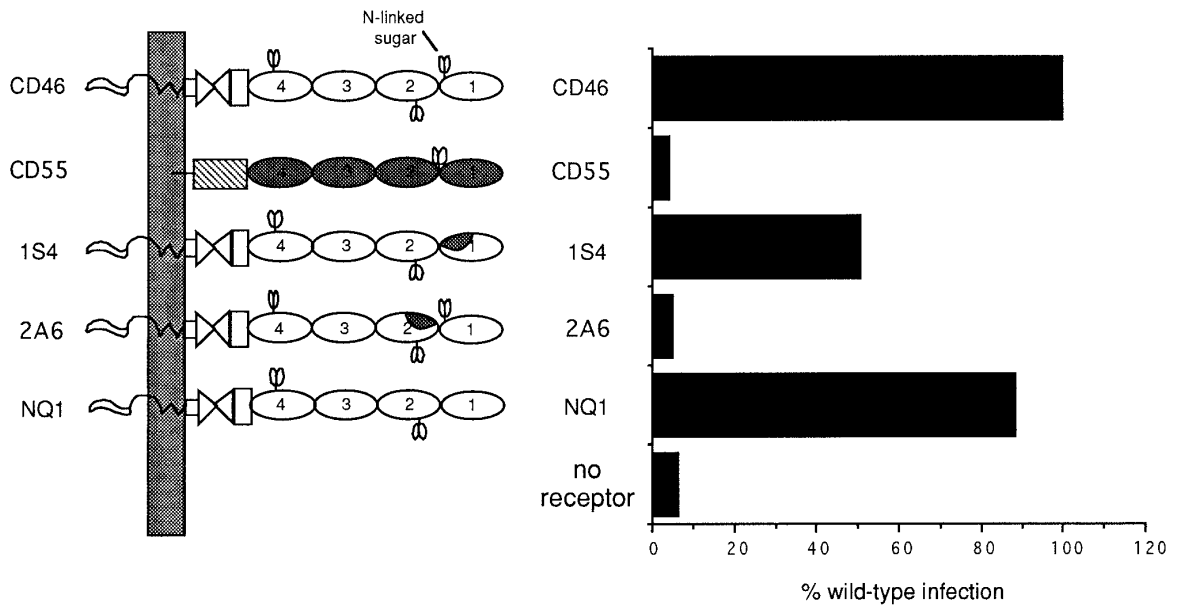
FIG. 4. Mutations of CD46 regions corresponding to inhibitory peptides 12 and 24. Schematic diagram indicates wild-type CD46 and CD55 molecules and chimeras. Short consensus repeats are designated 1–4. Sites of N-linked glycosylation are indicated. For the 1S4 chimera, the shaded region in SCR1 indicates the sequence DRNHTWLPVSDDACY (amino acids 47–61) from CD46 SCR1 was replaced by the sequence LKGSQWSDIEEFCN from the corresponding region of SCR1 in CD55. For the 2A6 chimera, the shaded region in SCR2 indicates the sequence FGYQMHFICNEGYYLIGEEI (amino acids 85–104) from CD46 SCR2 was replaced by the sequence FPVGTVVEYECRPGYRREPSLSPK from the corresponding region of SCR2 in CD55. The NQ1 mutation substitutes glutamine for asparagine at amino acid 49 and thus removes the consensus signal for N-linked glycosylation in SCR1. MV infection of CHO cells alone (no receptor) or CHO cells expressing CD46 mutants or controls is shown on the right. CHO cell lines were sorted by FACS to generate cell populations with equivalent receptor expression on the surface. Cells were infected in parallel with MV (m.o.i. = 2) and percentage of infected cells quantitated at 24 hr postinfection. Percentage of infected cells is expressed as the mean value with standard error of less than 5% for all samples. Values are normalized for the amount of infection compared to wild type, set at 100%.

this position led to a minimal (12%) reduction in MV infection (Fig. 4), indicating that this oligosaccharide is not required for MV attachment to the receptor and confirming the results of Maisner et al. (1996).

When peptide 24 sequences from SCR2 were replaced by CD55 sequences, a 94% reduction in MV infection was seen (Fig. 4). This is similar to the level of MV infection of CHO cells (no receptor, 93% reduction from wild-type) CHO cells expressing CD55 alone (97% reduction). This is also comparable to the reduced amount of MV infection seen when CD46 SCR2 is completely removed (Manchester et al., 1995). The profound loss of receptor function seen with this chimera is consistent with the inhibition seen with peptide 24 both in the peptide inhibition assay and in the hemagglutination assay (Figs. 1–3). Again these results confirm the role of this region of SCR2 in interacting with MV.

Structural modeling of MV binding domains on CD46

An amino acid sequence alignment was made between CD46 SCRs 1-2 and factor H SCRs 15-16. These two regions have 42% amino acid similarity (data not shown). Using the known NMR structure of factor H SCRs 15-16 as a guide (Barlow et al., 1993), the SCRs 1-2 of CD46 were modeled using the program O (Jones et al., 1991) (Figs. 5A and 5B). A stereo view (A) and space-filling model (B) of CD46 SCR1-2 are shown in Fig. 5. Orientation of the two modules relative to each other was consistent with that of the factor H structure (Barlow et al., 1993). In this orientation, identification of the two MV binding domains (peptide 12, amino acids 37–56 of SCR1, and peptide 24, amino acids 85–104 of SCR2) depicted in blue indicate that these two regions are on the surface and lie on the same face of the molecule.

FIG. 5. Model of CD46 with MV binding regions. (A) A stereoview of the ribbon diagram of the CD46 model. Amino (N) and carboxyl (C) termini are indicated. Regions corresponding to peptides 12 (SCR1, upper module) and 24 (SCR2, lower module) are color-coded in blue and the rest of the model in red. The ribbon diagrams were generated by the program RIBBONS (Carson, 1991). (B) Space-filling drawing of the CD46 SCR1-2 model with the same color scheme as in (A), with all atoms shown as spheres of 1.8 Å. The MV binding regions corresponding to peptides 12 and 24 shown in blue lie on the same face of the molecule. The space-filling drawing was made with the program MidasPlus (Ferrin et al., 1988; Huang et al., 1991). (C) Schematic model of CD46 on the cell surface interacting with MV glycoproteins on the virion membrane. MV-binding regions are depicted in blue. SCRs, N-linked oligosaccharides, and cytoplasmic regions of CD46 are indicated. MV H and F glycoproteins are depicted on the virion surface.

A
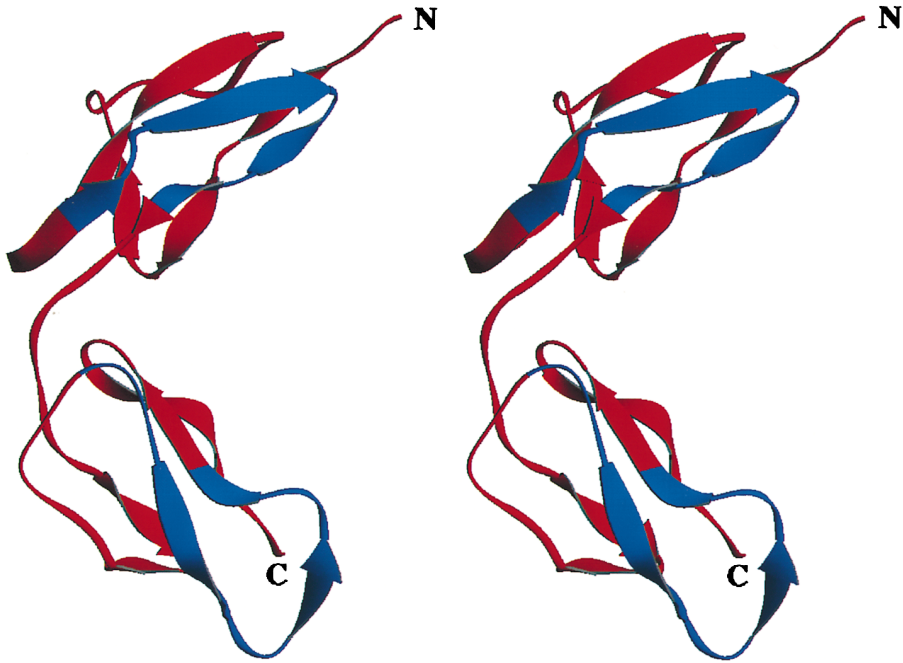
B

C
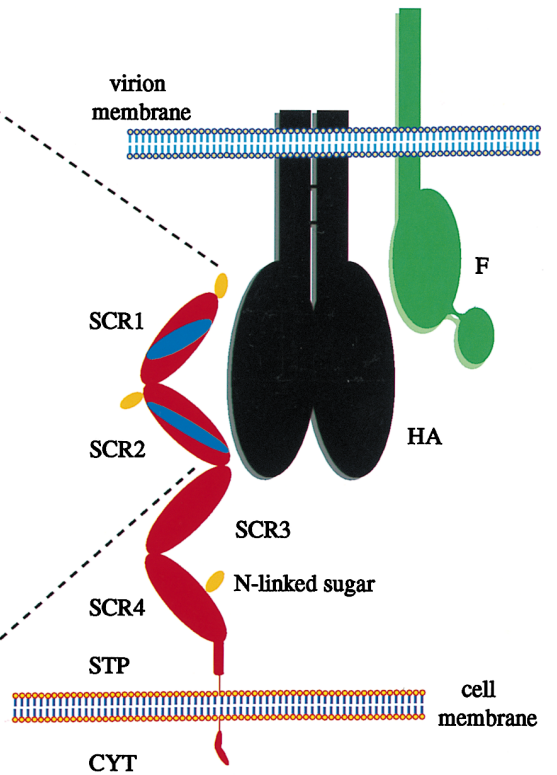
DISCUSSION

Here we further define regions of CD46 SCR1 and SCR2 that are involved in MV binding and infection. The combination of peptide mapping and mutagenesis studies demonstrate that MV contacts at least two distinct regions within SCR1-2 and that peptides corresponding to these regions inhibit MV infection. Our data also support the finding of Seya *et al.* (1995) and Devaux *et al.* (1996), showing that a soluble form of CD46 can block MV infection.

SCRs are a highly conserved structural motif among members of the RCA superfamily and are also found in many other proteins such as interleukin 2 receptor, factor XIIIb, and several viral proteins (Hourcade *et al.*, 1989). While structural information on CD46 is not available, solution NMR data on two SCRs from factor H shows the basic SCR structure to be a compact beta-barrel of approximately 15 by 30Å, with disulfide bonds between the first and third cysteines (C1–C3) and between the second and fourth cysteines (C2–C4) (Barlow *et al.*, 1991, 1992). The more conserved regions of the module form the β-barrel core, while the less well conserved regions generally form exposed regions. It is thought that all SCRs have this basic structure.

Mapping of the receptor–ligand interactions for other members of the RCA family suggest that most complement ligands interact with the exposed loops, rather than residues that make up the conserved hydrophobic core (Krych *et al.*, 1991, 1994; Molina *et al.*, 1994, 1995). Our data show that MV interacts with at least two regions that are most likely on the surface, but are not necessarily in nonconserved domains. These specific interactions with portions of SCR1-2 provide a means for MV to discriminate between CD46 and other complement receptors with similar structures. Although peptides can form secondary structure they may not assume the conformation that is found within the complete polypeptide. Thus MV may interact with additional structural determinants in SCR1-2 that peptide mapping studies cannot identify. Further mutagenic analysis may reveal other regions important for the MV–CD46 interaction that the peptide mapping has not.

What tertiary structure does the MV H recognize, and how are the SCRs arranged relative to one another? Analysis of single and paired SCRs from factor H show that SCRs are not arranged in a strictly linear fashion, but instead an SCR pair forms an angle of approximately 100°, with 131 ± 41° of twist about the long axis of the module (ω) for the second SCR of a pair relative to the first SCR (Barlow *et al.*, 1993; Molina *et al.*, 1995). It is predicted that for a particular pair of modules, the degree of angle and twist for adjacent SCRs will be unique and will be determined by the number and composition of the residues that form the interface between a particular SCR pair (Barlow *et al.*, 1993; Molina *et al.*, 1995). For

modeling of SCR1-2 of CD46, no additional conformational changes from factor H (tilt angle or degree of twist for the two SCRs relative to one another) were required to achieve proximity of the MV binding domains on the same face of the molecule. Based on the modeling studies of SCR1-2 we suggest that the modules are oriented relative to one another such that the peptide binding regions occupy positions on the same face of the molecule and that the module pair forms a binding pocket that allows MV H to dock against this region (Figs. 5A and 5B).

A model for the interaction of MV with CD46 is shown in Fig. 5C. The MV H and F proteins are shown on the virion membrane. While little is known about the structure of MV H it has been suggested that the CD46 binding domain lies within amino acids 451–505 (Hummel and Bellini, 1995), with particular contribution by amino acids 451 and 481 (Lecouturier *et al.*, 1996). While CD46 is depicted here as a monomer on the cell surface this has not yet been directly determined. Nevertheless, Devaux *et al.* have shown that when studying purified proteins in solution one dimer of MV H interacts with one monomer of CD46 (Devaux *et al.*, 1996).

Maisner *et al.* (1994; Maisner and Herrler, 1995) showed that MV binding to CD46 is dependent upon *N*-glycans, but that these *N*-glycans need not be complex oligosaccharides. These findings suggest the oligosaccharides contribute to conformation of the virus binding site but not specifically to recognition by MV. Interestingly, while the oligosaccharide in SCR1 is not required for MV binding, the oligosaccharide in SCR2 is required for binding (data not shown and Maisner *et al.*, 1996). The oligosaccharide in SCR2 may be required for maintaining the conformational angle or reducing flexibility between SCR1-2 and therefore providing a more rigid docking site for MV H.

Despite 40 million infections per year, no specific anti-MV therapeutic strategies exist. Our studies indicate use of small molecules mimicking CD46 may be an effective strategy for anti-MV therapies. For example, peptides 12 and 24, although their blocking activity is only in the millimolar range, possibly represent starting candidates for the development of more potent therapeutic molecules. MV infection is typically self-limiting, but complications such as pneumonia and immunosuppression enhance morbidity and mortality (Griffin and Bellini, 1996). Karp *et al.* (1996) have shown that ligand binding to CD46 induces downregulation of IL-12 production by monocytes, potentially leading to immunosuppression. This IL-12 downregulation might be avoided by using molecules that block the MV–CD46 interaction. Finally, it has been proposed that the interaction between MV H and CD46 that leads to downmodulation of CD46 from the cell surface also renders cells more susceptible to complement deposition and lysis (Krantic *et al.*, 1995; Schneider-Schaulies *et al.*, 1995, 1996; Schnorr *et al.*, 1995). Re-

agents that specifically block the MV H–CD46 interaction might also block downmodulation and preserve cells from complement-mediated lysis without otherwise perturbing the complement system.

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