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Functional Studies of Truncated Soluble Intercellular Adhesion Molecule 1 Expressed in *Escherichia coli*

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We have expressed in *Escherichia coli* the two N-terminal immunoglobulin (Ig)-like domains of the intercellular adhesion molecule 1 (ICAM-1). The first 188 residues of ICAM-1 were expressed with an N-terminal methionine (MP188) or as a maltose-binding fusion protein which was cleaved with factor Xa (XP188). After refolding, both MP188 and XP188 were active in binding to the leukocyte integrin lymphocyte function-associated antigen 1, which has previously been shown to bind to the N-terminal Ig domain of ICAM-1. The major group of rhinoviruses and malaria-infected erythrocytes bind to distinct sites within the first Ig-like domain of ICAM-1. Both MP188 and XP188 bound to malaria-infected erythrocytes; however, only XP188 inhibited human rhinovirus plaque formation. A product (MdQ1P188) with the initiation methionine fused to residue 2, i.e., with glutamine 1 deleted, inhibited plaque formation. MdQ1P188 was able to induce a conformational change of the virus capsid as shown by conversion of 149S particles to 85S particles, whereas MP188 had no effect. These results show that functionally active fragments of ICAM-1 can be produced in *E. coli*, that glycosylation is not required for ligand binding, and that the N-terminal residue of ICAM-1 is proximal to or part of the human rhinovirus-binding site.

The intercellular adhesion molecule 1 (ICAM-1) consists of five extracellular immunoglobulin (Ig)-like domains, a transmembrane-spanning region, and a short intracellular domain (24, 27). ICAM-1 serves as the counter receptor for the leukocyte integrins lymphocyte function-associated antigen 1 (LFA-1) and Mac-1 (25). ICAM-1 is also utilized as a receptor for the major group of human rhinoviruses (HRV) (5, 18, 28, 32); the binding site is located in the N-terminal extracellular domain (12, 26). A soluble recombinant ICAM-1 molecule (sICAM-1) containing all five extracellular Ig-like domains (11) and, more recently, a shorter ICAM-1 truncation containing 185 N-terminal amino acids (the first two Ig-like domains) (6) have been shown to prevent HRV infections in vitro. These soluble ICAM-1 molecules were produced in eukaryotic cells. Here we describe high-level expression of the N-terminal two domains (188 residues) of ICAM-1 in *Escherichia coli*. After refolding and purification with monoclonal antibodies (MAb), binding sites for LFA-1 and malaria-infected erythrocytes were functional, whereas binding to HRV depended on the amino-terminal sequence used to obtain expression in *E. coli*.

MATERIALS AND METHODS

Antibodies. ICAM-1 MAb RR1/1 (19) and MAb R6.5 (20) and LFA-1 MAb TS1/22 (22) have previously been described.

Expression of proteins. All ICAM-1 truncations were created by polymerase chain reactions (PCR). To produce pMalc-XP188, the pMalc vector (16) was cut with *Stu*I and *Hin*dIII and ligated with a PCR fragment produced by a 5′ PCR primer (5′-CAGACATCTGTGTCCCCCTCA-3′), encoding the first seven amino acids of ICAM-1, and a 3′ PCR primer (5′-CAGCTCCAGACCTTTGTCCTGCCATGAAAG CTTTTT-3′), containing the coding sequence for eight amino acids ending with proline 188, a translational stop codon, and a *Hin*dIII restriction site. The 5′ end of the ICAM-1 PCR fragment was inserted in the pMalc vector directly after a sequence encoding Ile-Glu-Gly-Arg, the protease recognition site for coagulation factor Xa.

To produce pET-MP188, the pET3a vector (17) was cut with *Nde*I and *Bam*HI and ligated to a fragment made with a 5′ PCR primer containing an *Nde*I restriction site, a methionine initiation codon, and the coding sequence for seven amino acids of ICAM-1 (5′-TTTCATATGCAGACATCT GTGTCCCCCTCA-3′) and a 3′ PCR primer with the coding sequence for eight amino acids ending with proline 188, a stop codon, and a *Bam*HI restriction site (5′-CAGCTCCA GACCTTTGTCCTGCCATGAGGATCCTTT-3′). To produce pET-MdQ1P188, the same 3′ PCR primer and a similar 5′ primer lacking the CAG codon for glutamine at position 1 were used. The correct sequence of the 5′ region of each construct was verified by sequencing. After the work described here was completed, a conservative Val-for-Phe substitution at residue 9 was discovered in MdQP188. This did not affect XP188 or MP188, since they were made in different PCR. Since MdQP188 but not MP188 binds rhinovirus, this mutation does not affect the conclusions of this study.

The constructs were transfected into the appropriate *E. coli* host strains: pMalc-XP188 in TB1 (7), pET-MP188 in BL-21, and pET-MdQ1P188 in both BL-21 and BL-21/DE3 (30). Large-scale production of XP188 was done by modification of a previously described procedure (16). Cells transformed with pMalc-XP188 were grown in Luria broth (LB) medium containing 1% glucose and 100 μg of ampicillin per ml to an optical density at 600 nm of 0.5 and then stimulated with 0.3 mM isopropyl-β-D-thiogalactoside (IPTG) for 6 h. Cells were harvested by centrifugation, resuspended in 50 ml of lysis buffer (10 mM Na_2HPO_4, 30 mM NaCl, 0.25% Tween 20, 10 mM β-mercaptoethanol, 10 mM EDTA [pH 7]) per liter of cell culture, and frozen at −20°C. After the cell suspension was thawed at 4°C, 1 mg of lysozyme per ml was added, and the mixture was held for 30 min on ice and then

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sonicated. The bacterial lysate was adjusted to 0.5 M NaCl and centrifuged for 30 min at 9,000 × *g* at 4°C. The supernatant was diluted 1:5 with column buffer (10 mM Na_2HPO_4, 0.5 M NaCl, 1 mM NaN_3, 10 mM β-mercaptoethanol [pH 7.2]) containing 0.25% Tween 20, loaded on an amylose column (New England Biolabs, Beverly, Mass.), and eluted as described previously (16). Protein-containing fractions were pooled and dialyzed against factor Xa buffer (20 mM Tris-HCl, 100 mM NaCl, 2 mM $CaCl_2$, 1 mM NaN_3) for 24 h at 4°C. Cleavage of the fusion protein was performed at room temperature for 3 days with 0.3 U of factor Xa per μg of fusion protein.

MP188 and MdQ1P188 were obtained in inclusion bodies. *E. coli* BL-21 containing pET-MP188 was grown in LB medium, and the recombinant protein was expressed without any stimulation. pET-MP188 and pET-MdQ1P188 transformants in *E. coli* BL-21/DE3 were grown to an optical density at 600 nm of 1.5 and then stimulated with 1 mM IPTG for 2 h. (There was no difference in antirhinoviral activity of MP188 obtained from BL-21 and BL-21/DE3.) For both transformants, bacteria were harvested by centrifugation and inclusion bodies were isolated as described previously (1). Cells from 1 liter of an overnight culture were centrifuged and resuspended with 1 min of sonication at maximum power in 100 ml of lysis buffer (25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.4], 12.5 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 0.1% Nonidet P-40, 20% glycerol, 0.5 M LiCl, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium metabisulfite), centrifuged for 15 min at 9,000 × *g*, resuspended in 100 ml of inclusion buffer 1 (20 mM Tris [pH 8.0], 20 mM EDTA) containing 1 mg of lysozyme per ml, and incubated for 15 min on ice. The cell lysate was then sonicated for 5 min at maximum power and centrifuged for 15 min at 9,000 × *g*. The pellet containing the inclusion bodies was washed twice in inclusion buffer 2 (10 mM Tris [pH 7.4], 1 mM EDTA, 0.5% Nonidet P-40, 1 mM dithiothreitol, 0.5 M LiCl) and once in inclusion buffer 3 (same as inclusion buffer 2 without LiCl) and sonicated to resuspend the inclusion bodies after each wash. The inclusion bodies were resuspended in 20 ml of H_2O.

Refolding. A matrix of refolding protocols with various protein concentrations, pHs, ratios of reducing-oxidizing substances, volumes of dialysis solution, and times was tested; for each protein, one optimal refolding protocol was developed.

After cleavage with factor Xa, XP188 was denatured by the addition of solid guanidine-HCl to make a 6 M solution. The solution was diluted to a final concentration of 100 μg/ml in 3 M guanidine-HCl–10 mM Tris HCl (pH 6.8), and 20 mM β-mercaptoethanol was added. After 2 h at 37°C under a N_2 atmosphere, the protein solution was dialyzed against 25 volumes of 10 mM Tris (pH 6.8) at 4°C, with several changes of the dialysis solution.

MP188 and MdQ1P188 inclusion bodies in 20 ml of H_2O were pelleted, resuspended in 20 ml of 6 M guanidine-HCl, and diluted 1:100 in 3 M guanidine–20 mM Tris-HCl (pH 8). Yields for a 1:20 dilution were much lower, and at pH 7, the yields were twofold lower. Reduced (1 mM) and oxidized (1 mM) glutathionine was added for 2 h at 37°C. The protein solution was dialyzed against 1 volume of 10 mM Tris-HCl (pH 8) for 16 h at room temperature and then dialyzed against 10 volumes at 4°C with several changes of the dialysis fluid. The gradual removal of guanidine was important.

Refolding was measured by testing different dilutions of the recombinant protein in a capture enzyme-linked immunosorbent assay (ELISA) (20) that used MAb RR1/1 to capture and biotinylated MAb R6.5 for detection and therefore requires renaturation of two separate MAb epitopes. Refolding yields of 15 to 20% were obtained with the protocols described above.

Refolded proteins were purified by immunoaffinity chromatography with ICAM-1 MAb R6.5-Sepharose CL-4B and high-pH elution (11). Final protein purification was performed by high-pressure liquid chromatography (HPLC; sizing column, TSK G3000-SW; Nest Group, Southboro, Mass.) for MP188 and MdQ1P188. Fast protein liquid chromatography (FPLC; sizing column, Superdex 75; Pharmacia) was used for XP188. Both sizing columns were run with phosphate-buffered saline (PBS; pH 7.4), protein containing fractions were pooled, and the volume was reduced by centrifugation with Centricon 30. Proteins were stored at concentrations of 1 to 2 mg/ml in PBS at 4°C.

Recombinant ICAM-1 produced in eukaryotic cells. A truncated sICAM-1 containing five domains was purified from CHO cell supernatants (11). A recombinant baculovirus was made to express an sICAM-1 fragment containing the two amino-terminal domains of ICAM-1 (ICI-2D) in insect (SF9) cells. A translational stop codon and *Xba*I site were introduced into ICAM-1 at the codon for L200 by oligonucleotide-directed mutagenesis with the primer 5'-CAGCCCCCGGGTCTAGAAGGTGGACACGC-3', as described previously (9, 26). The *Xba*I fragment containing sequence coding for IC1-2D was subcloned into the baculovirus transfer vector pBluebac (Invitrogen, San Diego, Calif.). Recombinant baculovirus expressing IC1-2D was isolated from plaques of SF9 cells cotransfected with the transfer vector construct and wild-type baculovirus DNA (31). IC1-2D was purified by immunoaffinity chromatography and by size exclusion chromatography from cultures of SF9 cells at 4 days postinfection with recombinant baculovirus (31).

Protein concentration. The protein concentration was determined by using a concentration of 0.8 mg/ml at an optical density of 280 nm, as calculated from the amino acid composition.

Binding assays. Recombinant ICAM-1 fragments (20 μg/ml for sICAM and 50 μg/ml for XP188 and MP188) in 25 μl of PBS were spotted onto 60-mm-diameter plastic petri dishes for 1 h at 37°C to obtain approximately 300 binding sites per μm^2 (3). The density was measured by saturation assay with ^{125}I-labeled MAb R6.5 as described previously (4). To measure binding to LFA-1, the dishes were incubated with 4×10^6 JY B lymphoblastoid cells in 2 ml, and after being washed, the bound cells were counted by using phase microscopy (3). Malaria-infected erythrocytes (2×10^7) with a parasitemia of 30 to 40% (33) were added in 2 ml, and after the cells were washed, the number of erythrocytes bound per square millimeter was determined (29).

Virus assay. Inhibition of HRV plaque formation was performed as described previously (23). Briefly, HeLa cells (3×10^5) were seeded in 60-mm-diameter tissue culture dishes in 5 ml of modified Eagle's medium with Earle's salts containing 10% fetal calf serum and nonessential amino acids at 37°C in 5% CO_2. After 48 h, the medium was removed, and the cell monolayer was washed with PBS. HRV type 3 (HRV-3) (200 PFU) was preincubated at 24°C for 30 min with different recombinant ICAM-1 fragments in 200 μl of PBS (pH 7.4) containing 1% fetal calf serum and added to HeLa cell monolayers. After 30 min at 24°C, 5 ml of agarose was added, and the monolayers were incubated for 48 h at 35°C in 5% CO_2, fixed with 4% formaldehyde, and after removal

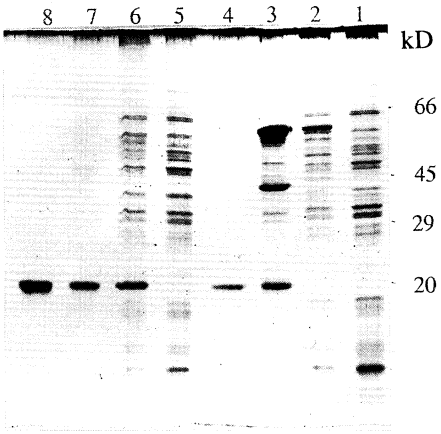

FIG. 1. ICAM-1 expressed in *E. coli*. Lysates of *E. coli* TB1 were transfected with the maltose-binding protein fusion construct (pMalc-XP188) without (lane 1) and with (lane 2) IPTG stimulation after amylose-affinity chromatography and factor Xa cleavage (lane 3) and after refolding and purification by ICAM-1 immunoaffinity chromatography and sizing column chromatography (lane 4). Lysates of *E. coli* BL-21 alone (lane 5) or transformed with pET-MP188 (lane 6) were analyzed. After refolding, MP188 was subjected to ICAM-1 immunoaffinity chromatography (lane 7) and HPLC purification (lane 8). Lysates from pelleted bacteria (equivalent to 1 μl of culture) or purified proteins (20 μl) were subjected to SDS–15% PAGE and silver staining. Size markers (in kilodaltons) are indicated on the right.

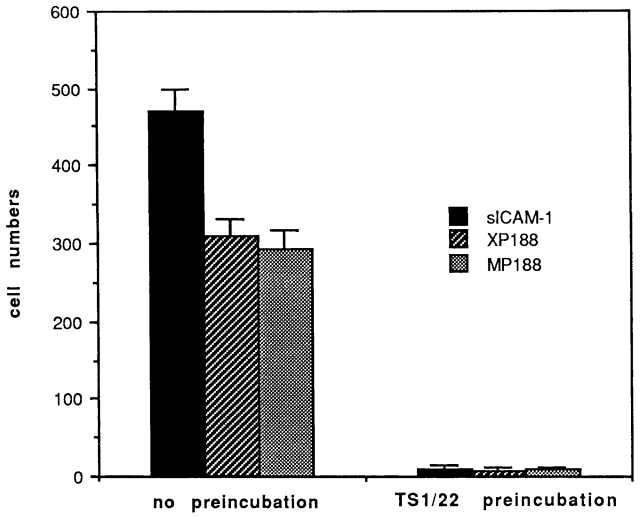

FIG. 2. Binding of LFA-1-positive cells (JY) to immobilized ICAM-1 fragments. JY cells bound to ICAM-1 fragments (300 sites per μm^2) were counted under the microscope (three different 1-mm^2 fields). Preincubation with blocking LFA-1 MAb (TS1/22) was performed for 15 min at 37°C. Error bars show the standard deviations for three experiments.

of agarose overlays, stained with 0.1% crystal violet. Plaques were counted, and the percent inhibition was calculated.

Assays of HRV conformation. HRV 14 was labeled with [^{3}H]leucine as described previously (21). The ability of ICAM-1 fragments to induce viral capsid conformation changes was examined by density gradient centrifugation. After preincubation of HRV with ICAM-1 preparations in a volume of 50 μl for 30 min at 37°C, samples were sedimented (40,000 rpm in an SW55 rotor, 1.5 h, 4°C) on a 5-ml sucrose gradient (5 to 30% in PBS with 0.01% bovine serum albumin [BSA]). The gradient was fractionated from the bottom into 200-μl fractions, and the ^{3}H radioactivity was determined.

RESULTS

The two most N-terminal Ig-like domains of ICAM-1 were expressed in two distinct systems. In the pMalc vector, the 188 N-terminal residues of ICAM-1 were fused to the C terminus of the maltose-binding protein. The fusion protein has an M_r of 62,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1, lane 2) and was purified by amylose-affinity chromatography. Cleavage with factor Xa resulted in a 42-kDa protein representing the maltose-binding protein and the 20-kDa ICAM-1 protein fragment (XP188) (lane 3). Some uncleaved material was still present. After refolding, XP188 was purified by immunoaffinity chromatography on R6.5 ICAM-1 MAb-Sepharose (lane 4) and a size exclusion column. A comparison with molecular weight standards showed that XP188 eluted in gel filtration with the size expected for a monomeric two-domain protein. The best recovery of functionally active, refolded protein was 0.1 mg/liter of bacterial cell culture.

The MP188 product was expressed in the pET3a vector by adding an initiation methionine to residues 1 to 188 of ICAM-1. MP188 was expressed in inclusion bodies and had an M_r of 20,000 (Fig. 1, lane 6). MP188 was approximately 90% of the inclusion body protein. Refolded MP188 was purified by R6.5 immunoaffinity chromatography (Fig. 1, lane 7), and approximately 10 mg of immunoreactive protein per liter of bacterial culture was recovered. Final purification was performed by size exclusion HPLC (lane 8). Its elution position showed that MP188 was monomeric. Refolding efficiency was monitored in a capture ELISA (20) with two ICAM-1 MAb (RR1/1 and R6.5) that recognize the native protein. Epitopes for RR1/1 and R6.5, located in the first and second domains, respectively, (26), must therefore be intact.

Recovery of MP188 after concentration with Centricon 30 cartridges was less than expected relative to BSA. Concentrations >2 mg/ml were difficult to obtain, and precipitation occurred. Freezing of the concentrated solutions also resulted in precipitation. Deglycosylation with N-glycosidase F of the five-domain sICAM-1 at 4 mg/ml produced in insect cells resulted in precipitation of completely deglycosylated material (data not shown). Solubility problems have not been noted with a two-domain form of ICAM-1 produced in CHO cells (6) or insect cells (data not shown). These results suggest that glycosylation is important for the solubility of ICAM-1.

The *E. coli*-produced ICAM-1 fragments XP188 and MP188 were tested for binding to LFA-1, which primarily recognizes the first Ig-like domain of ICAM-1. LFA-1-positive JY lymphoblastoid cells bound to both XP188 and MP188 (Fig. 2). Preincubation of cells with blocking LFA-1 MAb (TS1/22) abrogated binding completely. XP188 and MP188 were about 70% as active in this assay as sICAM-1, which contains all five Ig-like domains of ICAM-1.

The binding site for erythrocytes infected with *Plasmodium falciparum* is also located in the first extracellular domain of ICAM-1 (14). XP188 and MP188 immobilized to plastic bound infected erythrocytes (Fig. 3), indicating the presence of a functional binding site. The binding activity of MdQ1P188 (see below) was identical to that of XP188 and MP188 (data not shown).

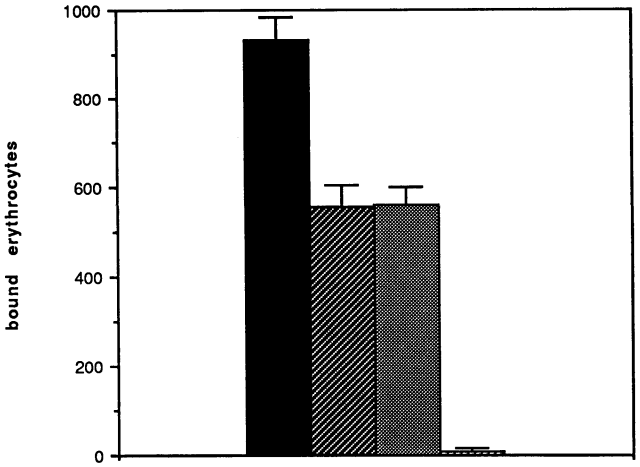

FIG. 3. Binding of *P. falciparum*-infected erythrocytes to immobilized sICAM-1 (■), XP188 (▨), and MP188 (▨). ICAM-1 fragments or, as a control, BSA (▨) was coated to plastic as described for JY cell binding. Erythrocytes (2×10^7, 30 to 40% parasitemia) were incubated for 20 min at 37°C. The plates were washed, and the number of erythrocytes bound per square millimeter was determined. Error bars indicate the standard deviations for three experiments.

The effectiveness of bacterial ICAM-1 fragments as antiviral agents was tested in a plaque reduction assay. HeLa cell monolayers were infected with HRV-3 preincubated with different concentrations of XP188 or MP188, and plaques were counted (Fig. 4). Surprisingly, XP188 but not MP188 inhibited HRV infection. The ICAM-1 fragments differ by the methionine added to the N terminus of MP188.

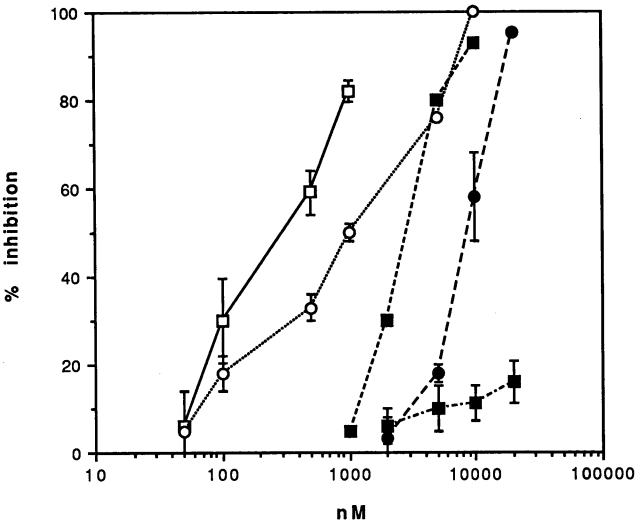

FIG. 4. Inhibition of HRV infectivity by ICAM-1 fragments. HRV-3 was preincubated with inhibitors as described in Materials and Methods and added to HeLa cell monolayers. After 48 h, plaques were counted and the percent inhibition was calculated. Controls gave 180 to 220 PFU. Datum points are the means and standard deviations (error bars) for four to five experiments (sICAM-1 [□]), two experiments (IC1-2D [○]), one experiment (results) (XP188 [■-----■]), two to four experiments (MdQ1P188 [●]), or three experiments (MP188 [■-···■]).

MP188 expressed in *E. coli* BL-21 or induced with IPTG in *E. coli* BL-21/DE3 was inactive (data not shown).

Since the extended amino terminus may interfere with ICAM-1 binding to HRV-3, MP188 was modified by deleting the second amino acid, glutamine (yielding MdQ1P188). MdQ1P188 was refolded under the same conditions and yielded immunoreactive protein in quantities similar to that of MP188. By contrast to MP188, MdQ1P188 inhibited plaque formation (Fig. 4). As reported previously (6), sICAM-1 fragments containing five extracellular domains were 10 times more active in prevention of HRV infections in vitro than fragments containing two Ig domains. Inhibition by XP188 was similar to or less than that of a two-domain fragment produced in insect cells (IC1-2D), whereas MdQ1P188 was three times less efficient.

Induction by ICAM-1 preparations of a conformational change in HRV-14 was tested by density gradient centrifugation. Sedimentation analysis of HRV (Fig. 5A) showed a single peak at fractions 5 and 6, representing intact virus (149S). Incubation with MdQ1P188 resulted in the appearance of a peak at fractions 13 and 14 (~85S), disappearance of the 149S peak, and appearance of a peak at fraction 7 (~120S) at intermediate concentrations (Fig. 5C). We have found that the ~120S peak contained ICAM-1 bound to HRV and was infectious and that the ~85S peak was not infectious (data not shown). MP188 at 20 μM did not induce a conformational change in HRV (Fig. 5B). The area under the 149S and ~120S peaks was integrated, and the reduction in these peaks was calculated as the percentage of conformational change (Fig. 5D). The five-domain sICAM-1 was the most potent inhibitor tested. A conformational change of 50% was achieved by 3 μM MdQ1P188. MdQ1P188 was similar in efficacy to the two-domain IC1-2D fragment produced in insect cells.

DISCUSSION

We have produced fragments containing the N-terminal 188 amino acids of ICAM-1 in *E. coli* that function in binding to LFA-1, to malaria-infected erythrocytes, and, depending on the N terminus, to HRV. XP188 was produced after factor X cleavage of a maltose-binding fusion protein expressed as a soluble protein in the cytoplasm of *E. coli*. Alternatively, an initiation methionine was joined to amino acids 1 to 188 or 2 to 188 of ICAM-1 (MP188 and MdQ1P188, respectively), resulting in expression of proteins in inclusion bodies. The final yield after refolding and MAb purification was much greater for MP188 and MdQ1P188, 10 mg/liter of culture, as opposed to 0.1 mg/liter for XP188. The greater quantities available allowed MP188 and MdQ1P188 to be more extensively characterized. A previous study of prokaryotic ICAM-1 limited to Western blotting (immunoblotting) of crude bacterial lysates containing ICAM-1 fragments fused to β-galactosidase showed reactivity with MAb to domain five but not with MAb to other regions, including domains 1 and 2 of ICAM-1 (13).

The refolded protein that we isolated by MAb affinity chromatography was in the correct conformation according to several criteria. The R6.5 MAb used for purification recognizes a conformation-sensitive determinant (26). The protein was active in a capture ELISA utilizing conformation-sensitive MAb that bind to distinct epitopes in the first and second Ig-like domains. Furthermore, the conformation-sensitive binding sites for LFA-1 (26), for malaria-infected erythrocytes (14), and in some constructs for HRV (26) were intact.

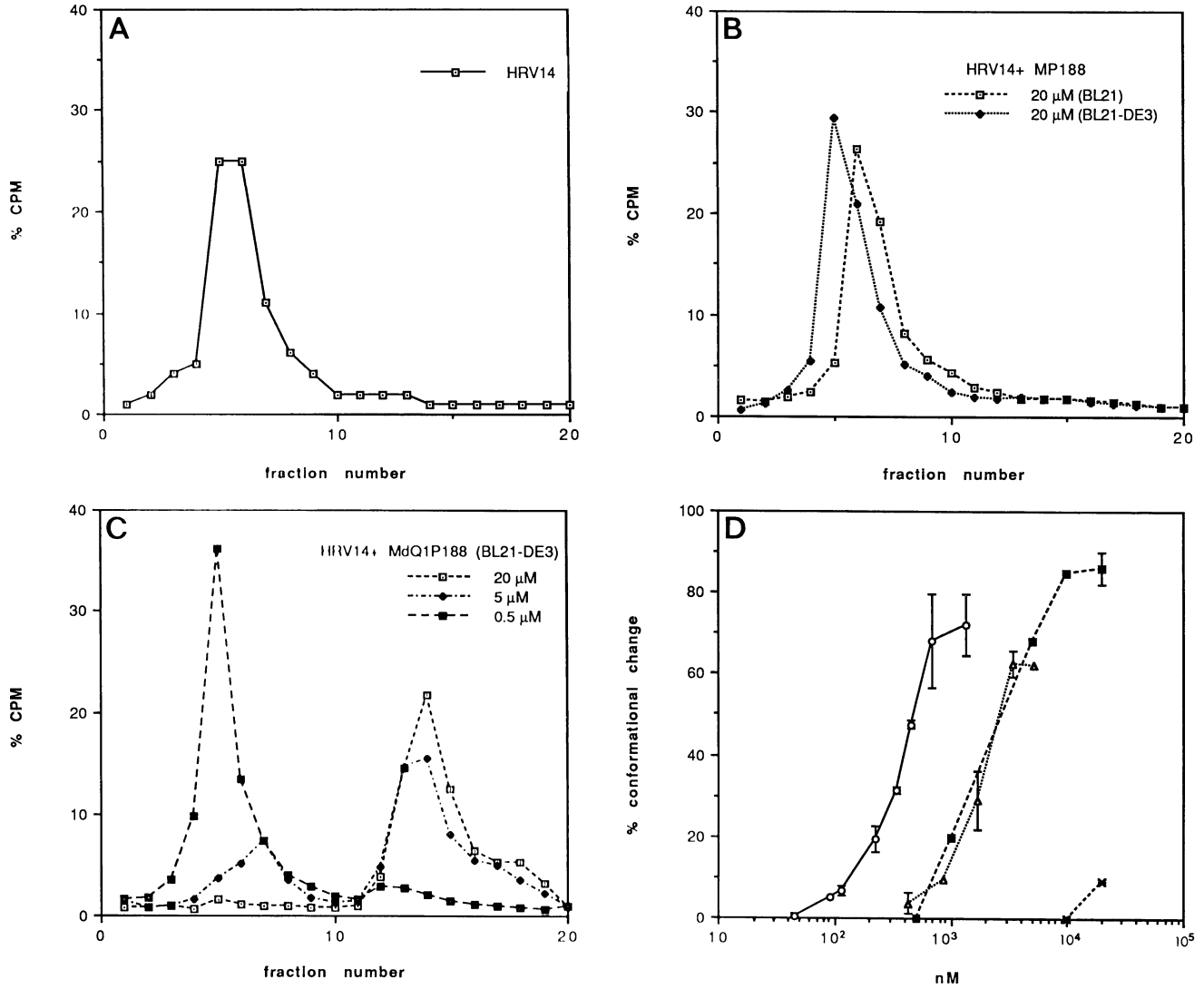

FIG. 5. Induction by ICAM-1 preparations of HRV-14 conformational change measured by density gradient centrifugation. ^{3}H-labeled HRV-14 with or without preincubation for 30 min at 37°C with ICAM-1 preparations was analyzed by centrifugation on a 5 to 30% (wt/vol) sucrose gradient. (A to C) Representative gradients were fractionated from the bottom into fractions of 250 μl, except for the last two fractions, which contained 600 μl, and ^{3}H radioactivity was measured. The 149S HRV-14 peak was in fractions 5 and 6. (D) After incubation with different concentrations of ICAM-1 preparations and density gradient sedimentation, the areas under the 149S and ~120S peaks (see panels A to C) were integrated. The reduction of the 149S and ~120S particles was calculated as the percentage of conversion to 85S particles (conformational change). Datum points are means ± standard deviations for three experiments (sICAM-1 [○]) or one or two experiments (IC1-2D [△], MdQ1P188 [X], and MP188 [■]).

The lack of N-linked glycosylation of proteins expressed in *E. coli* appears to be responsible for the weak solubility of MP188. The first two domains of ICAM-1 contain four N-linked glycosylation sites (27). Deglycosylation of ICAM-1 produced in eukaryotic cells resulted in precipitation at similar concentrations. These findings suggest that N-linked glycans on ICAM-1 mask hydrophobic patches on the protein surface and are required for solubility. The poor solubility of MP188 hindered attempts at crystallization for structural analysis, which requires concentrations above 10 mg/ml. A crystal structure for the first two Ig-like domains of CD4 was obtained with a fragment produced in *E. coli* (34); however, no N-linked glycosylation sites are present in this region of CD4.

The ability of ICAM-1 to inhibit HRV infection (11) and to induce a conformational change in the virus (6) was demonstrable for XP188 but not for MP188. The induction of a conformational change in rhinovirus by binding to a receptor results in the loss of viral RNA from the capsid (6) and is likely to be closely associated with the mechanism of RNA entry into the cytosol during infection of a cell. The N-terminal methionine present in MP188 seems to interfere with the binding site for HRV, as shown with two different serotypes, HRV-3 and HRV-14. N-terminal sequencing of MP188 revealed that the methionine had not been posttranslationally removed in *E. coli* but had been deformylated (10a). The receptor-binding site on HRV has been identified as a deep surface depression, or "canyon" (2, 18). Mutagenic analysis of ICAM-1 (12, 15, 26) located the binding site to domain 1 of ICAM-1. The N terminus has been shown to

be involved in binding since mutation of Gln-1–Thr-2 to Lys-1–Ala-2 reduces rhinovirus binding by 10-fold (26).

To examine the effect of the length of the N-terminal segment on binding to rhinovirus, Gln-1 was deleted in the protein MdQ1P188. MdQ1P188 is the same length as XP188, differing only in substitution of Met for Gln at the N terminus. MdQ1P188 was active in the prevention of HRV infection of HeLa cells and induction of conformational change in the virus capsid. The ability of MdQ1P188 and XP188 but not MP188 to bind rhinovirus strongly suggests that the N-terminal methionine in MP188 interferes sterically with binding to rhinovirus.

Our data indicate that glycosylation is not required for the interaction of ICAM-1 with LFA-1, malaria-infected erythrocytes, or HRV. This is consistent with the results of McClelland et al. (12), who deleted by site-directed mutagenesis the four N-linked sites individually, but not altogether, and found no effect on binding to rhinovirus. These results contrast with those of another report (10) that described a crucial role for glycosylation in binding to rhinovirus, on the basis of in vitro translation in the absence and presence of dog microsomes.

After incubation with purified receptor proteins, picornaviruses such as poliovirus (8) and rhinovirus (6) undergo a capsid conformational change with the subsequent loss of viral RNA. Preincubation of HRV for 0.5 h with MdQ1P188 induced a conformational change in the virus capsid, demonstrated by the loss of 149S particles and the appearance of ~85S particles. These data extend previous results (6) by showing that two-domain fragments of ICAM-1 can efficiently induce this conformational change. This change results in irreversible loss of viral infectivity and thus is the most profound mechanism of virus neutralization.

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REFERENCES

1. **Bohmann, D., and R. Tjian.** 1989. Biochemical analysis of transcriptional activation by Jun: differential activity of c- and v-Jun. Cell **59:**709–717.
2. **Colonno, R. J., J. H. Condra, S. Mizutani, P. L. Callahan, M. E. Davies, and M. A. Murcko.** 1988. Evidence for the direct involvement of the rhinovirus canyon in receptor binding. Proc. Natl. Acad. Sci. USA **85:**5449–5453.
3. **Diamond, M. S., D. E. Staunton, S. D. Marlin, and T. A. Springer.** 1991. Binding of the integrin Mac-1 (CD11b/CD18) to the third Ig-like domain of ICAM-1 (CD54) and its regulation by glycosylation. Cell **65:**961–971.
4. **Dustin, M. L., and T. A. Springer.** 1989. T cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. Nature (London) **341:**619–624.
5. **Greve, J. M., G. Davis, A. M. Meyer, C. P. Forte, S. C. Yost, C. W. Marlor, M. E. Kamarck, and A. McClelland.** 1989. The major human rhinovirus receptor is ICAM-1. Cell **56:**839–847.
6. **Greve, J. M., C. P. Forte, C. W. Marlor, A. M. Meyer, H. Hoover-Litty, D. Wunderlich, and A. McClelland.** 1991. Mechanisms of receptor-mediated rhinovirus neutralization defined by two soluble forms of ICAM-1. J. Virol. **65:**6015–6023.
7. **Johnston, T. C., R. B. Thompson, and T. O. Baldman.** 1986. Nucleotide sequence of the *luxB* gene of *Vibrio harveyi* and the complete amino acid sequence of the β subunit of bacterial luciferase. J. Biol. Chem. **261:**4805–4811.
8. **Kaplan, G., M. S. Freistadt, and V. R. Racaniello.** 1990. Neutralization of poliovirus by cell receptors expressed in insect cells. J. Virol. **64:**4697–4702.
9. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82:**488–492.
10. **Lineberger, D. W., D. J. Graham, J. E. Tomassini, and R. J. Colonno.** 1990. Antibodies that block rhinovirus attachment map to domain 1 of the major group receptor. J. Virol. **64:**2582–2587.

10a. **Marlin, S.** Personal communication.

11. **Marlin, S. D., D. E. Staunton, T. A. Springer, C. Stratowa, W. Sommergruber, and V. Merluzzi.** 1990. A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection. Nature (London) **344:**70–72.
12. **McClelland, A., J. DeBear, S. C. Yost, A. M. Meyer, C. W. Marlor, and J. M. Greve.** 1991. Identification of monoclonal antibody epitopes and critical residues for rhinovirus binding in domain 1 of intercellular adhesion molecule 1. Proc. Natl. Acad. Sci. USA **88:**7993–7997.
13. **Mölgg, M., W. Schwaeble, J. P. Johnson, and M. P. Dierich.** 1991. Generation of recombinant, carbohydrate-free intercellular adhesion molecule-1 (ICAM-1) and ICAM-1 fragments in *Escherichia coli* and mapping of epitopes recognized by anti-ICAM-1 monoclonal antibodies. Immunol. Lett. **28:**237–244.
14. **Ockenhouse, C. F., R. Betageri, T. A. Springer, and D. E. Staunton.** 1992. Plasmodium falciparum-infected erythrocytes bind ICAM-1 at a site distinct from LFA-1, Mac-1, and human rhinovirus. Cell **68:**63–69.
15. **Register, R. B., C. R. Uncapher, A. M. Naylor, D. W. Lineberger, and R. J. Colonno.** 1991. Human-murine chimeras of ICAM-1 identify amino acid residues critical for rhinovirus and antibody binding. J. Virol. **65:**6589–6596.
16. **Riggs, P.** 1990. Expression and purification of maltose-binding protein fusions, p. 16.6.1–16.6.12. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. Greene Publishing Associates and Wiley Interscience, New York.
17. **Rosenberg, A. H., B. N. Lade, D.-S. Chui, S.-W. Lin, J. J. Dunn, and F. W. Studier.** 1987. Vectors for selective expression of cloned DNAs by T7 RNA polymerase. Gene **56:**125–135.
18. **Rossmann, M. G.** 1989. The Canyon hypothesis: hiding the host cell receptor attachment site on a viral surface from immune surveillance. J. Biol. Chem. **264:**14587–14590.
19. **Rothlein, R., M. L. Dustin, S. D. Marlin, and T. A. Springer.** 1986. A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. J. Immunol. **137:**1270–1274.
20. **Rothlein, R., E. A. Mainolfi, M. Czajkowski, and S. D. Marlin.** 1991. A form of circulating ICAM-1 in human serum. J. Immunol. **147:**3788–3793.
21. **Rueckert, R. R., and M. A. Pallansch.** 1981. Preparation and characterization of encephalomyocarditis virus. Methods Enzymol. **78:**315–325.
22. **Sanchez-Madrid, F., J. Nagy, E. Robbins, P. Simon, and T. A. Springer.** 1983. A human leukocyte differentiation antigen family with distinct alpha subunits and a common beta subunit: the lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p150,95 molecule. J. Exp. Med. **158:**1785–1803.
23. **Sherry, B., and R. Rueckert.** 1985. Evidence for at least two dominant neutralization antigens on human rhinovirus 14. J. Virol. **53:**137–143.
24. **Simmons, D., M. W. Makgoba, and B. Seed.** 1988. ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. Nature (London) **331:**624–627.
25. **Springer, T. A.** 1990. Adhesion receptors of the immune system. Nature (London) **346:**425–433.
26. **Staunton, D. E., M. L. Dustin, H. P. Erickson, and T. A. Springer.** 1990. The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding sites for LFA-1 and

rhinovirus. Cell **61:**243–254.
27. **Staunton, D. E., S. D. Marlin, C. Stratowa, M. L. Dustin, and T. A. Springer.** 1988. Primary structure of intercellular adhesion molecule 1 (ICAM-1) demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell **52:**925–933.
28. **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:**849–853.
29. **Staunton, D. E., C. F. Ockenhouse, and T. A. Springer.** 1992. Soluble intercellular adhesion molecule 1-immunoglobulin G1 immunoadhesion mediates phagocytosis of malaria-infected erythrocytes. J. Exp. Med. **176:**1471–1476.
30. **Studier, F. W., and B. A. Moffatt.** 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. **189:**113–130.
31. **Summer, M. D., and G. E. Smith.** 1988. A manual of methods for baculovirus vectors and insect cell culture procedures, p. 1–57. Texas Agricultural Experiment Station, College Station, Tex.
32. **Tomassini, J. E., D. Graham, C. M. DeWitt, D. W. Lineberger, J. A. Rodkey, and R. J. Colonno.** 1989. cDNA cloning reveals that the major group rhinovirus receptor on HeLa cells is intercellular adhesion molecule 1. Proc. Natl. Acad. Sci. USA **86:**4907–4911.
33. **Trager, W., and J. B. Jensen.** 1976. Human malaria parasites in continuous culture. Science **193:**673–675.
34. **Wang, J., Y. Yan, T. P. J. Garrett, J. Liu, D. W. Rodgers, R. L. Garlick, G. E. Tarr, Y. Husain, E. L. Reinherz, and S. C. Harrison.** 1990. Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. Nature (London) **348:**411–418.