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Antibodies That Block Rhinovirus Attachment Map to Domain 1 of the Major Group Receptor

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The vast majority of human rhinovirus serotypes utilize the intercellular adhesion molecule 1 (ICAM-1) as the attachment site on susceptible cells. Twelve murine monoclonal antibodies were isolated and shown by competition binding studies to recognize three distinct, nonoverlapping epitopes on the ICAM-1 receptor. Titration of three antibodies representing each of the binding sites demonstrated that they were equally effective at blocking viral attachment. By using in vitro transcription and translation systems, a series of progressive C-terminal truncations of ICAM-1 molecules was generated. Immunoprecipitation of these fragments with each of the three antibodies indicated that all three epitopes reside within the first 82 amino acids of the receptor. Attempts to demonstrate specific binding of these in vitro-synthesized receptor fragments to virions were unsuccessful. The inability to show virion binding was most likely due to a failure of the lysates to properly glycosylate the receptor molecule, since native, unglycosylated receptor molecules isolated from cell membranes were also inactive in virus binding assays.

Human rhinoviruses (HRVs), members of the family *Picornaviridae*, are the major causative agents of the common cold, one of the more elusive diseases known to humans (10). It has been shown in earlier studies that HRVs initiate infection by binding to one of two different receptors on susceptible cells (3). Of the 101 serotypes tested, 91 competed for a single cellular receptor and were designated the "major" group, while the remaining 10 serotypes competed for a second receptor and comprised the "minor" group (1, 4; unpublished data). We have previously reported the isolation of an antireceptor monoclonal antibody (MAb) (designated MAb 1A6) that specifically blocks attachment of the major HRV group (4). The major group viruses exhibit an absolute requirement for this receptor, since blocking attachment with MAb 1A6 was cytoprotective despite high titer viral challenge (4). In vivo chimpanzee and human clinical trials involving MAb 1A6 have also demonstrated that this receptor is involved in HRV infection of the nasal cavity (6, 11).

By using MAb 1A6 and immunoaffinity chromatography, a 90-kilodalton (kDa) glycoprotein was isolated from HeLa cell membranes and was subsequently shown to be the receptor protein required for the attachment of the major group HRVs to susceptible cells (23). Biochemical characterization of the purified 90-kDa receptor protein determined that it was an acidic glycoprotein and contained seven N-linked glycosylation sites (25). Recent cDNA cloning experiments have identified the receptor as the intercellular adhesion molecule 1 (ICAM-1) (9, 22, 24). ICAM-1 is a cell surface ligand for the lymphocyte function-associated antigen 1 adhesion receptor (15) and is a member of the immunoglobulin supergene family, since it is predicted to contain five homologous immunoglobulinlike domains (20). Some sequence homology exists between ICAM-1 and other adhesion proteins of the adult nervous system, namely, neural cell adhesion molecule and myelin-associated glycoprotein (20, 21). The interaction of ICAM-1 and the lymphocyte function-associated antigen 1 plays an important role in leukocyte adhesion and in the execution of immunological and inflammatory functions mediated by leukocyte adhesion (7).

In order to define the regions of ICAM-1 that interact with antireceptor antibodies and virions, cell-free transcription and translation systems were utilized to generate several unique fragments of ICAM-1. Characterization of these fragments indicates that the N-terminal domain of ICAM-1 is important for MAb binding and suggests that this portion of the receptor is crucial for virus binding.

MATERIALS AND METHODS

Antibodies. The isolation and characterization of murine MAb 1A6 has been previously described (4). To generate additional antibodies, BALB/c mice were immunized with the immunoaffinity-purified ICAM-1 receptor (23) and hybridomas were prepared as described previously (4). The resulting hybridoma supernatants were screened in a cell protection assay (4). Eleven positive hybridoma supernatants were identified, and their corresponding cells were subcloned by limiting dilution to generate individual colonies. MAbs were isolated from each resulting cell line and were purified by protein A and fast protein liquid chromatography (11). MAbs were iodinated and used in the competition binding assays described in Table 1. Hybridoma cells producing OKT4 MAb were obtained from the American Type Culture Collection, and antibody was isolated from ascitic fluid as described previously (11).

Generation of ICAM-1 fragments. The 5′-terminal noncoding region and the entire coding region of the ICAM-1 cDNA clone, pHRVr1 (24), was excised by digestion with restriction enzymes *Eco*RI and *Hin*dIII and was then ligated into the transcription vector pGEM4Z, which was digested with the same enzymes to form pGEM-ICAM. A full-length RNA copy of ICAM-1 was subsequently generated by digestion of pGEM-ICAM with *Hin*dIII and subsequent transcription in vitro according to the procedure of the manufacturer (Promega Biotec). Similarly, successive truncations of ICAM-1 were generated by digesting the plasmid with *Bal*I, *Nar*I, *Mae*I, and *Rsa*I, respectively, and then transcribing each in vitro. Each RNA was extracted with phenol-chloro-

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TABLE 1. Competition binding of MAbs

Radiolabeled MAb	% Binding[a]			
	Blocking MAbs			
	None	1A6	2C2	18B9
1A6	42	7	37	38
2C2	43	43	10	42
18B9	33	39	34	7

[a] Confluent HeLa cell monolayers (48-well cluster plate) were preincubated with 1 μg of the indicated MAb or media in a total volume of 0.1 ml for 30 min at 37°C. Radiolabeled MAbs in 50 μl of media were then added to each well, and incubation was continued for 1 h. Values shown represent the percentage of I^{125} radioactivity associated with cell monolayers after removal of media and washing of the monolayer with phosphate-buffered saline as previously described (4).

form and chloroform and then was precipitated with ethanol in the presence of 2.75 M ammonium acetate.

A new construct of the domain 1 fragment was created by polymerase chain reaction amplification as follows. DNA was primed at the 5′ end by an oligonucleotide (5′-ACGCC AGGGTTTTCCCAGTCACGA-3′) homologous to the sense strand and located upstream of the SP6 promoter and was primed at the 3′ end with an antisense oligonucleotide (5′-CATCATCATCATGTACACGGTGAGGAAGGTTT TAGC-3′) homologous to ICAM-1 cDNA and containing 12 additional bases encoding four Met residues. The polymerase chain reaction was performed as described by the supplier with a DNA Thermal Cycler (Cetus-Perkin-Elmer).

Translation of ICAM-1 RNAs. Precipitated RNA was suspended in water at 1 mg/ml and stored at −20°C until it was used in translation reactions. Translation reaction mixtures, in a 0.1-ml final volume, contained 0.02 mM of each essential amino acid except methionine, 0.05 mg of calf liver tRNA per ml, 800 U of RNasin per ml, 7.5 mCi of [^{35}S]methionine per ml (Amersham Corp.), 2 μg of each protease inhibitor (pepstatin A, antipain, and leupeptin) per ml, 16 U of canine pancreatic microsomal membranes per ml at an optical density of 280 nm (Dupont, NEN Research Products), 0.6 ml of rabbit reticulocyte lysate per ml of reaction mixture, 2 mM oxidized glutathione (added just prior to the addition of mRNA), and 5 μg of mRNA. Reactions were carried out at 30°C for 30 to 60 min, stopped by the addition of ribonuclease A to 0.1 mg/ml, and then incubated an additional 10 min at 30°C. Radiolabeled protein was analyzed by autoradiography of translated products following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Immunoprecipitation of translation products. Following translation, the reaction mixtures were cooled on ice, phenylmethylsulfonyl fluoride was added to 2 mM, and membrane vesicles were disrupted by adding Nonidet P-40 to 0.5%. Twenty microliters of a 50% slurry of protein A-agarose (Boehringer Mannheim Biochemicals) was added, and the mixture was rotated at 4°C for 10 min. The mixtures were pelleted (2 min in a microcentrifuge at 5,000 rpm), and samples of the supernatants were transferred to tubes containing 0.5 μg of the indicated MAb and were brought to a final volume of 0.1 ml with Tris-buffered saline containing 0.25% Nonidet P-40. Following immunoprecipitation overnight at 4°C, 20 μl of protein A-agarose was added and this material was mixed for 30 min at 4°C while rotating. The agarose beads were gently pelleted and then washed twice with Tris-buffered saline containing 0.5% Nonidet P-40 and once with Tris-buffered saline alone. Pelleted agarose beads were suspended in 2× Laemmli protein gel loading buffer (13) and were incubated at 100°C for 3 min; eluted proteins were then analyzed by SDS-PAGE and fluorography.

Preparation of native and deglycosylated ICAM-1. Native receptor was prepared by inducing ICAM-1 expression in confluent HeLa cell monolayers with 2,000 U of gamma interferon per ml and 10 ng of tumor necrosis factor per ml of RPMI(-Met) medium (GIBCO) containing 2.5% dialyzed fetal calf serum. [^{35}S]methionine was added to 100 μCi/ml, and the cells were incubated overnight at 37°C. Unglycosylated ICAM-1 was prepared from cells pretreated for 4 h with tunicamycin (1 μg/ml) prior to labeling as described above. The resulting receptor proteins were purified by affinity chromatography as previously described (23).

Virus binding assay. The binding of HRVs to purified receptor protein has been described elsewhere (9). Briefly, [^{35}S]methionine-labeled receptor protein was incubated with gradient-purified HRV-36 or HRV-2 virions in 0.1 ml of N buffer (10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.5], 150 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% Triton X-100) at 37°C for 30 min. The reaction mixture was chilled on ice, layered onto a 0.15-ml cushion of 10% glycerol–2 M triethanolamine (pH 7.5) in Beckman airfuge tubes, and centrifuged at 134,000 × *g* for 30 min at 4°C. Five 50-μl fractions were removed from the top, and the virus pellet was then suspended in 50 μl of 1% SDS buffer. Samples of each fraction were then assayed for radioactivity. Material in the last fraction and resuspended pellet fractions were pooled and analyzed by radiography following SDS-PAGE.

RESULTS

Isolation and characterization of antireceptor MAbs. The previous finding that MAb 1A6 was very effective in blocking virus attachment and infection suggested that this MAb most likely binds to a region of ICAM-1 that is in close proximity to the HRV binding site. Similar studies with the CD4 receptor, which serves as a binding site for human immunodeficiency virus (HIV), have used MAbs that block virus binding to identify the binding site for gp120 (2, 14, 17). Since the ICAM-1 and CD4 receptors are both immunoglobulinlike structures, we proceeded to isolate a panel of 12 murine MAbs that block the attachment of viruses belonging to the major HRV group. Reciprocal competition binding studies resulted in the assignment of the 12 MAbs into three nonoverlapping binding groups (data not shown). One antibody from each group (1A6, 2C2, and 18B9) was selected for further analysis, and their inability to block the binding of each other in cell binding studies is documented in Table 1. In every case, each individual MAb was blocked by its homologous MAb and not by the other two heterologous MAbs. The residual binding observed in the homologous blocking experiments appears to be nonspecific in nature, since low-level binding can be observed with receptor negative cells (data not shown).

To determine whether one MAb binding site was more efficacious than the others, each of the three MAbs was titrated for its ability to inhibit virus binding to HeLa cell monolayers. All three MAbs were nearly identical in their ability to block HRV-36 attachment with 50% inhibitory concentrations of 180, 320, and 420 ng/ml for MAbs 1A6, 2C2, and 18B9, respectively (Fig. 1).

Immunoprecipitation of ICAM-1 fragments. The ICAM-1 receptor is a member of the immunoglobulin supergene family and is predicted to have five homologous immunoglo-

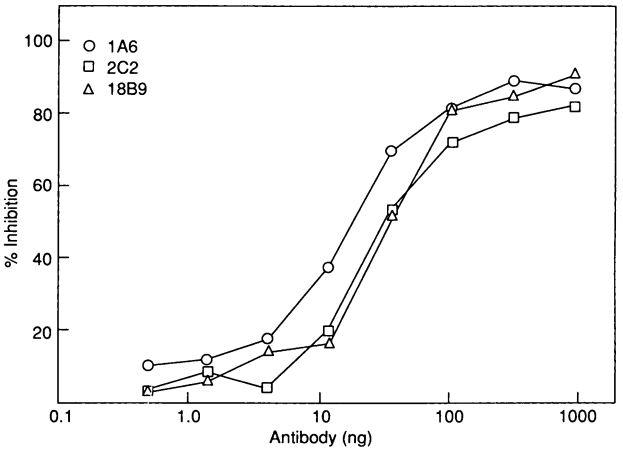

FIG. 1. Inhibition of HRV attachment by MAbs. Confluent HeLa cell monolayers in 48-well cluster plates were treated in duplicate with the indicated amounts of purified MAbs 1A6, 2C2, and 18B9 in 0.1 ml of media for 30 min at 34°C. Following incubation, a 50-μl solution containing [^{35}S]methionine-labeled HRV-36 was added to untreated and MAb-treated wells, and the cultures were incubated an additional hour at 34°C. The amount of unbound (supernatant) and bound (cell monolayer) virus was determined as described previously (1) and was used to calculate the percent inhibition of control binding.

bulinlike domains defined by amino acids 1 through 88, 89 through 185, 186 through 284, 285 through 385, and 386 through 453 (21). A schematic diagram of the ICAM-1 receptor is illustrated in Fig. 2. A full-length cDNA clone (pHRVr1) was shown to have a single, large, open reading frame initiating at nucleotide 72 that encodes 532 amino acids and contains a 1,333-nucleotide 3′ noncoding region (24). Signal and transmembrane sequences are predicted to exist near the N- and C-terminal regions of the molecule, respectively. A full-length subclone was inserted into a pGEM4Z transcription vector to form pGEM-ICAM. The

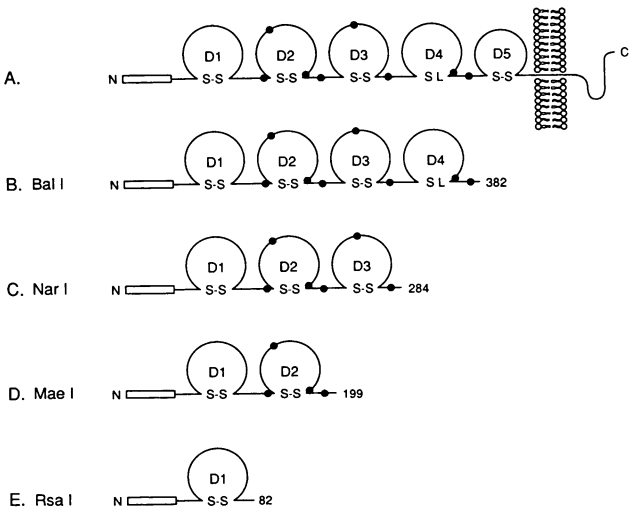

FIG. 2. Schematic diagram of ICAM-1 proteins generated by in vitro translation. Listed restriction enzymes indicate the sites used to truncate ICAM-1 cDNA. The numbers at the end of each line indicate the number of the last amino acid residue. The closed circles indicate the locations of putative N-linked glycosylation sites. D1, D2, D3, D4, and D5 represent domains 1 through 5, respectively. S-S, Proposed disufide; N, amino terminus; C, carboxyl terminus.

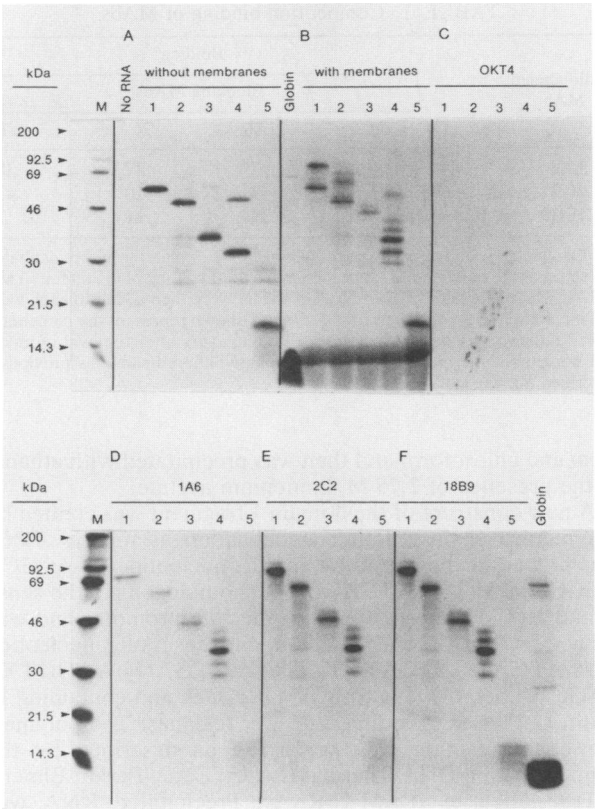

FIG. 3. Cell-free translation and immunoprecipitation of ICAM-1 polypeptides. In vitro-transcribed RNAs encoding full-length ICAM-1 protein (lane 1), domains 1 through 4 (lane 2), domains 1 through 3 (lane 3), domains 1 and 2 (lane 4), and domain 1 (lane 5) were translated in rabbit reticulocyte lysates in the absence (A) or presence (B) of added microsomal membranes as described in Materials and Methods. Translations in the presence of microsomal membranes were spiked with in vitro-translated globin and were subjected to immunoprecipitation with the indicated MAbs in panels C through F. All samples were analyzed by SDS-PAGE and autoradiography. The positions of protein markers and globin are as indicated. No RNA, Translation products generated in the absence of added mRNA.

orientation of the cloned sequence is such that the 5′ terminus is just downstream of the phage SP6 promoter. Thus, specific ICAM-1 mRNA can be transcribed from linearized plasmid DNA with SP6 RNA polymerase. Prior to transcription, plasmid DNA was cleaved with conveniently located restriction enzymes to enable generation of RNA fragments encoding selective domains of the ICAM-1 receptor molecule (Fig. 2).

In vitro translation of the resulting RNAs in rabbit reticulocyte lysates generated polypeptides of the expected molecular weights as determined by SDS-PAGE (Fig. 3A). In vitro-synthesized polypeptides were immunoprecipitated as an initial strategy to map the binding domains of each of the three MAbs. Despite repeated attempts, no specific immunoprecipitation of the core proteins could be demonstrated with any of the MAbs (data not shown). Since the ICAM-1 receptor is a membrane protein and has been shown to contain seven N-linked glycosylation sites (25), canine microsomal membranes were added to the translation mixture in an attempt to optimize the folding of the ICAM-1 molecules by allowing them to be inserted through a membrane.

In addition, previous studies had demonstrated that adding oxidized glutathione to rabbit reticulocyte lysates containing microsomal membranes conferred proper conformation of pancreatic secretory proteins expressed in vitro (19).

Translation of full-length ICAM-1 mRNA in the presence of microsomal membranes and 2 mM oxidized glutathione resulted in two major bands with molecular sizes of approximately 55 and 70 kDa (Fig. 3B, lane 1). The higher-molecular-weight band is presumably the result of core glycosylation activity associated with microsomal membranes (8), although this species remains significantly smaller than the 90-kDa size observed for the native receptor. Larger species were also observed with each of the other ICAM-1 RNA species, except the domain 1 fragment which continued to generate a 16-kDa polypeptide (Fig. 3B). This result was expected, since the domain 1 amino acid sequence is devoid of potential glycosylation sites. Immunoprecipitation experiments, involving each of the ICAM-1 polypeptide fragments synthesized in the presence of microsomal membranes, were specifically precipitated by each of the MAbs (Fig. 3D through F). Radiolabeled proteins encoding polypeptides as short as 82 amino acids were still recognized by all three MAbs. Two specificity controls were incorporated into these experiments. The first involved the parallel testing of MAb OKT4, which recognizes the CD4 receptor; the second was the addition of radiolabeled globin to each immunoprecipitation sample. Both of these controls indicated that the immunoprecipitations were specific, since MAb OKT4 was unable to recognize the ICAM-1 polypeptides (Fig. 3C) and since globin was not immunoprecipitated by the anti-ICAM-1 MAbs (Fig. 3D through F). Because the immunoprecipitation efficiency of the domain 1 fragment was low when MAbs 1A6 and 2C2 were used, a new construct was created that incorporated additional methionine residues at the C-terminal end of this molecule (see Materials and Methods). Translation of this fragment generates a 16-kDa polypeptide with significantly greater radioactivity. Immunoprecipitation of this domain 1 fragment in the presence of a control protein (yeast alpha mating factor) resulted in a specific immunoprecipitation by the three anti-ICAM-1 MAbs and not by the OKT4 MAb (Fig. 4). This result confirms the initial observation that the three MAbs recognize epitopes within the first 82 amino acids of the ICAM-1 molecule.

Virus binding to purified ICAM-1 proteins. The finding that all three MAbs blocked HRV attachment and recognized sites within domain 1 of ICAM-1 implied that the binding site for major group HRVs is located at or near this region of the ICAM-1 protein. To test this directly, a virus binding assay was utilized that measures the ability of radiolabeled receptor molecules to cosediment with HRV-36, a major group serotype. To verify the binding specificity of the assay, the native receptor was isolated by immunoaffinity chromatography from HeLa cells that were cytokine induced in the presence of [^{35}S]methionine. Utilization of this material in a virus binding assay showed that 51% of the labeled receptor comigrated with HRV-36. SDS-PAGE analysis confirmed the presence of the 90-kDa ICAM-1 receptor in the pellet fractions (data not shown). The virus-receptor interaction was blocked by the addition of Fab fragments of MAb 1A6 and did not occur in the presence of HRV-2, a minor group HRV (Table 2).

Assay of in vitro-synthesized, full-length ICAM-1 and the polypeptide representing just domain 1, failed to show any specific interaction with HRV-36 (Table 2). The inability to bind to virus could be due to the inability of the ICAM-1 molecule to assume the proper configuration or to the absence of specific glycosylation. To test the latter, HeLa cell monolayers were treated with tunicamycin to block glycosylation and radiolabeled receptor was isolated as before. Immunoaffinity isolation of the resulting ICAM-1 protein produced the expected 55-kDa receptor molecule previously described (25). Assay of the unglycosylated ICAM-1 molecule in the virus binding assay also failed to show any interaction with virus. These results taken together suggest that glycosylation plays an important role in virus binding either directly or through a conformational alteration of the mature protein.

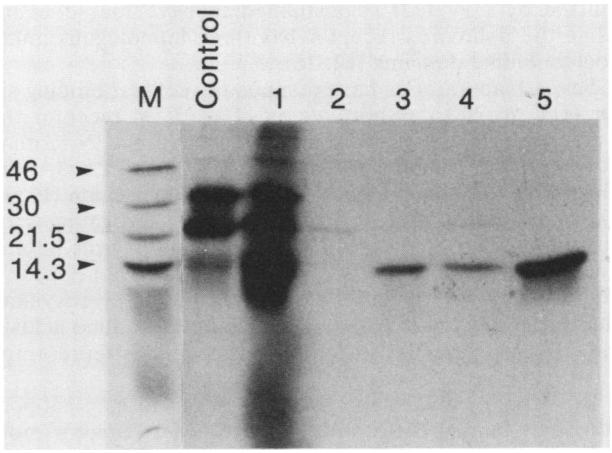

FIG. 4. Cell-free translation and immunoprecipitation of domain 1. In vitro-transcribed RNA resulting from the polymerase chain reaction-amplified modification of the domain 1 fragment was translated in the presence of added microsomal membranes and then spiked with a translated yeast alpha mating protein (Promega Biotec) prior to immunoprecipitation and SDS-PAGE analysis. The control lane shows the position of yeast alpha mating protein alone, while lane 1 shows the control protein and ICAM-1 domain together. The material generated in lane 1 was utilized in immunoprecipitations with MAbs OKT4 (lane 2), 1A6 (lane 3), 2C2 (lane 4), and 18B9 (lane 5). The positions of low-molecular-weight markers (in thousands) are displayed in lane M.

DISCUSSION

HRVs initiate infection of cells by attaching to specific cellular receptors. In the case of the major group of HRVs, this receptor has been identified as the adhesion ligand, ICAM-1 (9, 22, 24). Transfection of cDNA encoding ICAM-1 into receptor-negative Vero cells results in a Vero cell line that will not only bind major group HRVs but will also support their replication (24; unpublished data). A series of MAbs has been isolated that efficiently abrogate attachment of the major group of HRVs and are cytoprotective. Further analysis of three of these antireceptor MAbs indicates that they bind to nonoverlapping sites on ICAM-1 and are equally effective at blocking virus attachment (Table 1; Fig. 1).

The prediction that ICAM-1 is structurally related to members of the immunoglobulin supergene family enables structural alignment of the ICAM-1 molecule with domains of other known members of the immunoglobulin supergene family. As a result of such alignments, ICAM-1 is predicted to have five homologous domains (20). The ICAM-1 receptor is, therefore, closely related in structure to the CD4 receptor and to the immunoglobulinlike protein recently identified as the cellular receptor for poliovirus (16). The CD4 receptor

utilized by HIV (12) is postulated to have four domains, while the poliovirus receptor has three homologous immunoglobulinlike domains (12, 16).

Several laboratories have mapped the cellular binding site for HIV gp120 to a short region of the CD4 receptor that resides within the first 53 amino acids of the N-terminal domain (12, 17). Anti-CD4 MAbs (OKT4A and Leu3A) that block HIV attachment also map to this same region (12, 14, 17) and suggest that MAbs capable of abrogating virus attachment can be utilized to map the site of virus interaction. Glycosylation of the CD4 receptor does not appear to play a functional role in HIV binding, since no glycosylation sites are predicted to exist within the first 53 amino acids of the CD4 receptor and since synthetic peptides representing this region block CD4-gp120 interaction (12).

By using a similar approach, we set out to map antibody and virus binding sites within the ICAM-1 receptor molecule. Our approach differed from previous studies in that we employed in vitro transcription and translation systems to generate fragments of the ICAM-1 receptor. Translation was further optimized by the inclusion of canine microsomal membranes and glutathione in an attempt to enhance the generation of biologically functional molecules. The microsomal membranes, which are only capable of core glycosylation, clearly altered the proteins synthesized in their presence. Immunoprecipitation experiments showed that only the proteins that had transversed the membranes were able to be immunoprecipitated with the MAbs (Fig. 3D through F). Interestingly, this was true for the shortest ICAM-1 fragment, which is not predicted to have glycosylation sites, and suggests that translation in the presence of microsomal membranes also has a conformational effect on in vitro-synthesized ICAM-1.

Experiments utilizing a subset of ICAM-1 polypeptides clearly showed that all three of the anti-ICAM-1 MAbs assayed were able to recognize the smallest fragment tested. This fragment represented the first 82 amino acids of the mature ICAM-1 molecule and encompassed only the first of five predicted domains. It is interesting to note that the 82-amino-acid-long fragment contains two potential sulfhydryl bonds bridging Cys residues (24) at positions 21 and 25 with Cys residues 65 and 69, respectively. These sulfhydryl bridges appear to be important in the conformation of domain 1, since shorter fragments deleting Cys residues 65 and 69 result in fragments no longer recognized by any of the MAbs (data not shown).

Mutagenic studies with an infectious cDNA clone of HRV-14 have yielded the best evidence to date that the virion attachment site is located within a 2.2-nm-deep crevice, or canyon, present on the surface of the virion (5). This canyon structure appears to be conserved among all of the HRVs, polioviruses, and coxsackieviruses B (18) and suggests that the cellular receptors for these viruses will most likely show some structural conservation (5). Computer modeling of ICAM-1 domain 1 proposes that the N-terminal half of domain 1 could occupy the HRV canyon and interact with amino acids located at the bottom of the canyon (V. L. Giranda, M. S. Chapman, and M. G. Rossmann, Proteins Struct. Funct. Genet., in press). Although highly speculative in nature, this model would support the MAb binding results obtained here that implicate domain 1 as the virus binding site.

Unlike studies with CD4, glycosylation appears to play an important role in the generation of a functional receptor protein capable of binding HRVs. Attempts to show specific virus interaction with either the full-length protein or the domain 1 fragment translated in vitro were unsuccessful (Table 2). Glycosylation appears to be more important for virus binding than for MAb binding, since unglycosylated native receptor isolated from HeLa cells by immunoaffinity chromatography fails to bind to HRV-36. The absence of carbohydrates could deter HRV binding directly, if it was itself part of the virus binding site, or indirectly, by causing an alternative conformation of the native protein that is incapable of efficient interaction with the virion attachment site. Interestingly, domain 1 of ICAM-1 contains none of the predicted glycosylation sites present in ICAM-1, while neighboring domains 2 and 3 are predicted to account for six N-linked glycosylation sites (Fig. 2).

TABLE 2. Virus binding to ICAM-1 proteins

Source (^{35}S-labeled receptor protein)	Presence of 1A6 Fab	% Radioactivity comigrating with virus[a]	
		HRV-36	HRV-2
Cell membranes (native [90 kDa])	−	51.0	4.5
	+	5.5	ND
Reticulate lysates (domains 1 through 5 [70 kDa])	−	5.1	7.1
	+	5.5	ND
Reticulate lysates (domain 1 [14 kDa])	−	3.9	4.3
	+	4.7	ND
Cell membranes (deglycosylated [55 kDa])	−	3.6	4.4
	+	4.5	ND

[a] Numbers reflect the percentage of total radioactivity present in the last fraction and resuspended pellet as described in Materials and Methods. ND, Not determined.

How the absence of carbohydrates in other domains of the native ICAM-1 receptor may affect the ability of domain 1 to interact with the virion canyon is unclear and could suggest a role for the glycosylated domains in the formation of the receptor multimers previously observed (23). Clearly, further characterization of the HRV–ICAM-1 interactions will help illuminate the role of carbohydrates and help define the conformation requirements necessary to generate a biologically active ICAM-1 receptor molecule.

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LITERATURE CITED

1. **Abraham, G., and R. J. Colonno.** 1984. Many rhinovirus serotypes share the same cellular receptor. J. Virol. **51**:340–345.
2. **Berger, E. A., T. R. Fuerst, and B. Moss.** 1988. A soluble recombinant polypeptide comprising the amino-terminal half of the extracellular region of the CD4 molecule contains an active binding site for human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **85**:2357–2361.
3. **Colonno, R. J.** 1987. Cell surface receptors for picornaviruses. Bioessays **5**:270–274.
4. **Colonno, R. J., P. L. Callahan, and W. J. Long.** 1986. Isolation of a monoclonal antibody that blocks attachment of the major group of human rhinoviruses. J. Virol. **57**:7–12.
5. **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.

6. **Colonno, R. J., J. E. Tomassini, and P. L. Callahan.** 1987. Isolation and characterization of a monoclonal antibody which blocks attachment of human rhinoviruses, p. 93–102. *In* M. A. Brinton and R. R. Rueckert (ed.), Positive strand RNA viruses. Alan R. Liss, Inc., New York.
7. **Dustin, M. L., D. E. Staunton, and T. A. Springer.** 1988. Supergene families meet in the immune system. Immunol. Today **9**:213–215.
8. **Goldman, B. M., and G. Blobel.** 1981. In vitro biosynthesis, core glycosylation, and membrane integration of opsin. J. Cell Biol. **90**:236–242.
9. **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.
10. **Gwaltney, J. M., Jr.** 1982. Rhinoviruses, p. 491–517. *In* E. A. Evans (ed.), Viral infection of man: epidemiology and control, 2nd ed. Plenum Publishing Corp., New York.
11. **Hayden, F. G., J. M. Gwaltney, Jr., and R. J. Colonno.** 1988. Modification of experimental rhinovirus colds by receptor blockade. Antiviral Res. **9**:233–247.
12. **Jameson, B. A., P. E. Rao, L. I. Kong, B. H. Hahn, G. M. Shaw, L. E. Hood, and S. B. H. Kent.** 1988. Location and chemical synthesis of a binding site for HIV-1 on the CD4 protein. Science **240**:1335–1339.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. **Landau, N. R., M. Warton, and D. R. Littman.** 1988. The envelope glycoprotein of the human immunodeficiency virus binds to the immunoglobulin-like domain of CD4. Nature (London) **334**:159–162.
15. **Makgoba, M. W., M. E. Sanders, G. E. Ginther Luce, E. A. Gugel, M. L. Dustin, T. A. Springer, and S. Shaw.** 1988. Functional evidence that intercellular adhesion molecule-1 (ICAM-1) is a ligand for LFA-1-dependent adhesion in T cell-mediated cytotoxicity. Eur. J. Immunol. **18**:637–640.
16. **Mendelsohn, C. L., E. Wimmer, and V. Racaniello.** 1988. Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. Cell **56**:855–865.
17. **Peterson, A., and B. Seed.** 1988. Genetic analysis of monoclonal antibody and HIV binding sites on the human lymphocyte antigen CD4. Cell **54**:65–72.
18. **Rossmann, M. G., and A. C. Palmenberg.** 1988. Conservation of the putative receptor attachment site in picornaviruses. Virology **164**:373–382.
19. **Scheele, G., and R. Jacoby.** 1982. Conformational changes associated with proteolytic processing of presecretory proteins allow glutathione-catalyzed formation of native disulfide bonds. J. Biol. Chem. **257**:12277–12282.
20. **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.
21. **Staunton, D. E., S. D. Marlin, C. Stratowa, M. L. Dustin, and T. A. Springer.** 1988. Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell **52**:925–933.
22. **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.
23. **Tomassini, J. E., and R. J. Colonno.** 1986. Isolation of a receptor protein involved in attachment of human rhinoviruses. J. Virol. **58**:290–295.
24. **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.
25. **Tomassini, J. E., T. R. Maxson, and R. J. Colonno.** 1989. Biochemical characterization of a glycoprotein required for rhinovirus attachment. J. Biol. Chem. **264**:1656–1662.