ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1992, p. 1186–1191
0066-4804/92/061186-06$02.00/0

Vol. 36, No. 6

In Vitro Studies of the Antirhinovirus Activity of Soluble Intercellular Adhesion Molecule-1

EURICO ARRUDA,[1]* CAROLYN E. CRUMP,[1] STEVEN D. MARLIN,[2] VINCENT J. MERLUZZI,[2] AND FREDERICK G. HAYDEN[1,3]

Departments of Internal Medicine[1] and Pathology,[3] Box 473, University of Virginia School of Medicine, Charlottesville, Virginia 22908,[1] and Department of Immunology, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, Connecticut 06877[2]

Received 23 December 1991/Accepted 13 March 1992

We studied the in vitro antirhinovirus activity of a soluble form of intercellular adhesion molecule-1 (sICAM-1). sICAM-1 inhibited the cytopathic effect of 10 representative human rhinovirus (HRV) serotypes of the major receptor group with, 50% effective concentrations (EC_{50}s) of 0.1 to 7.9 μg/ml. Cell type-dependent variation in the inhibitory activity of sICAM-1 was observed for two major receptor group serotypes in HeLa cells (EC_{50}, >32 μg/ml), and no inhibitory effect was observed for two serotypes which use different cell receptors. Yield reduction assays showed that sICAM-1 inhibited the replication of HRV serotype 39 (HRV-39) in human adenoid explants in a concentration-dependent manner. No direct inactivation of infectivity of HRV-39 (EC_{50}, 0.5 μg/ml) was observed after incubation with sICAM-1 (32 μg/ml) for up to 24 h. Single-cycle-of-replication experiments with the addition of sICAM-1 at 10 μg/ml at different times showed that the inhibitory effect occurs only when sICAM-1 is added within 30 min after infection. In experiments in which absorption was carried out at 4°C and then a single cycle of replication incubation was carried out at 33°C, it was found that sICAM-1 at 10 μg/ml was inhibitory only when it was present during the absorption period. Our data show that sICAM-1 is inhibitory for representative major receptor group serotypes of HRV in two cell lines and human respiratory epithelium, that the interaction of sICAM-1 with HRV is readily reversible by dilution, and that the inhibitory effect of sICAM-1 on virus replication is present early in the infection cycle.

The glycoprotein intercellular adhesion molecule type 1 (ICAM-1) has been identified as the cell receptor for the major receptor group of human rhinoviruses (HRVs), which includes approximately 90% of the numbered serotypes (7, 14, 15). ICAM-1, which is a member of the immunoglobulin superfamily, is a cell surface molecule which functions as a ligand for the lymphocyte function-associated antigen-1 and promotes interactions between leukocytes and a number of cell types (9). ICAM-1 is composed of five extracellular immunoglobulinlike domains, one transmembrane anchor, and an intracytoplasmic C-terminal domain (7, 14). The primary site for the interaction of both lymphocyte function-associated antigen-1 and HRV seems to be within domain 1 of ICAM-1 (13). Soluble forms of ICAM-1 (sICAM-1) have been expressed in Chinese hamster ovary (CHO) cells and have been shown to inhibit the cytopathic effect (CPE) caused by HRVs and other picornaviruses that use ICAM-1 as a cell receptor (8, 10). In one study (10), sICAM-1 concentrations of approximately 1 μg/ml (approximately 18 nM) inhibited the CPE of HRV serotype 54 (HRV-54) by 50% (50% inhibitory concentration), and 10 μg/ml inhibited the CPE by more than 90%. When binding of radiolabeled HRV-14 to HeLa cells was measured, concentrations of 50 μg/ml (approximately 0.9 μM) were necessary to inhibit binding by 50% (10). Another study (8) found that 0.4 μM sICAM-1 inhibited HRV-3 infectivity by 50% and that concentrations of about 3 μM caused 50% inhibition of radiolabeled HRV-3 binding to ICAM-1 immobilized on microtiter dishes. Here we report the results of in vitro studies conducted to assess further the antirhinovirus activity of sICAM-1 for representative major receptor group serotypes of HRV in cultures of established cell lines and explants of human respiratory epithelium and to assess the mechanism of antirhinovirus activity of sICAM-1.

MATERIALS AND METHODS

Viruses, cells, and reagents. Laboratory-passaged stocks of HRV-1A, -3, -5, -14, -16, -28, -35, -36, -39, -54, -59, -64, -66, and -87 were originally obtained from the American Type Culture Collection (Rockville, Md.). For mechanism of action studies, a stock of HRV-39 was grown in HeLa R19 cells. The cells were harvested when the monolayers exhibited a 100% CPE and were then frozen-thawed three times. The supernatants were clarified by centrifugation (250 × *g*) and precipitated with polyethylene glycol, and the virus was banded in a metrizamide gradient by published procedures (1). The infectious titer of the final virus pool was 8 $\log_{10}$ 50% tissue culture infective doses ($TCID_{50}$s) per ml.

HeLa R19 cells were a gift from Richard Colonno (Merck Sharp & Dohme, West Point, Pa.). Ohio HeLa cells were a gift from D. A. J. Tyrrell (Common Cold Unit, Medical Research Council, Salisbury, England), and WI-38 fibroblasts were purchased from Whittaker M. A. Bioproducts (Walkersville, Md.). Growth media were minimal essential medium with Earle's salts supplemented with 10% bovine (5% fetal plus 5% calf) serum (for HeLa cells) or with 10% fetal serum (for WI-38 fibroblasts), antibiotics, and 1% glutamine. Maintenance media were McCoy's medium supplemented with 30 mM $MgCl_2$ and 2% bovine fetal serum (for HeLa cells) or minimal essential medium with Earle's salts supplemented with 5% bovine fetal serum (for WI-38 fibroblasts).

sICAM-1 was obtained by immunoaffinity purification of supernatants of cultures of CHO cells that expressed the

* Corresponding author.

cloned cDNA (9, 12). The anti-ICAM-1 monoclonal antibody R6.5 was prepared and characterized as described previously (12, 14).

Pirodavir, a capsid-binding agent with antipicornavirus activity (4), was provided by K. Andries (Janssen Research Foundation, Beerse, Belgium).

Guinea pig neutralizing anti-HRV-39 antiserum was purchased from the American Type Culture Collection, and neutralizing anti-HRV-39 monoclonal antibody was a gift from Jack M. Gwaltney, Jr. (University of Virginia, Charlottesville).

Determination of the cytotoxicity of sICAM-1. The toxic effects of sICAM-1 on the logarithmic growth of cell cultures were determined for Ohio HeLa and WI-38 cells. Cells were seeded in 24-well plates at approximately 6×10^4 cells per well in medium with sICAM-1 at concentrations that varied from 0 to 100 μg/ml; quadruplicate wells were used for each concentration of sICAM-1. The cell cultures were incubated at 37°C in a CO_2 incubator and were examined daily for monolayer confluence and morphological signs of toxicity (ballooning, refractiveness, granularity, shrinkage), and counting of viable cells was done after the cells were stained with trypan blue on the third and sixth days after seeding.

Determination of effective inhibitory concentrations for rhinovirus serotypes of the major receptor groups. The ability of sICAM-1 to inhibit infection by 12 different HRV serotypes was tested in CPE inhibition assays performed in 96-well microtiter plates as described previously (3), with minor modifications. Quadruplicate monolayers of HeLa and WI-38 cells were used, concentrations of sICAM-1 varied between 0.1 and 32 μg/ml in 0.5 $\log_{10}$ dilutions, and the extent of the CPE in sICAM-1-exposed monolayers was determined when control monolayers showed 80 to 100% CPE, generally 3 to 4 days postinoculation. The 50% inhibitory concentration for the development of CPE (EC_{50}) was determined by using the software Dose-Effect Analysis with Microcomputers (Biosoft, Cambridge, United Kingdom). For each rhinovirus serotype tested, the monoclonal antibody R6.5 and the antiviral agent pirodavir (0.1 μg/ml) were used as positive controls.

Effect of sICAM-1 on the yield of HRV-39 in human respiratory epithelium. The inhibitory effects of a range of sICAM-1 concentrations on virus yield from multiple rounds of replication were tested in cell monolayers grown from explants of human adenoid epithelium. Adenoids removed from people undergoing adenoidectomy were transported to the laboratory in Hanks balanced salt solution with antibiotics. The procedure for the preparation of the explants and obtaining monolayer outgrowth around the explanted fragments was modified from previously published procedures (3, 17). Briefly, fragments were cut from the epithelial surface of the adenoid within 6 h after removal and were dissected to produce 2-mm^3 explants. The explants were placed into dry 24-well plastic dishes at five explants per well. The explants were left at room temperature to attach to the dishes for 20 min and were then covered with minimal essential medium with Earle's salts and D-valine (GIBCO-Bethesda Research Laboratories, Grand Island, N.Y.), 10% Nu-Serum (Collaborative Research, Lexington, Mass.), and antibiotics. Dishes were incubated at 33°C in an incubator with 5% CO_2, and the medium was changed every 3 days. The fragments were checked for the presence and extent of outgrowth and for the presence of ciliated cells. After 1 to 2 weeks of incubation, the explants that produced a semiconfluent monolayer, in which beating cilia were often seen, were selected for use. For the yield reduction assay, quadruplicate explant monolayers obtained from the same adenoid were exposed to 1,000 to 5,600 $TCID_{50}$s of HRV-39 or HRV-59 in the presence of sICAM-1 at concentrations of 0, 1, 3.2, or 10 μg/ml. After a 3-h absorption period at 33°C, the monolayers were washed four times with Hanks balanced salt solution and refed 1.0 ml of medium. At that point, in three experiments, monolayers that were exposed to no sICAM-1 during the absorption period were washed and refed the medium containing 10 μg of sICAM-1 per ml. At 24, 48, and 72 h after inoculation, the supernatant was collected and frozen for titration of virus yield in WI-38 fibroblast monolayers. After the collection of supernatants at 24 and 48 h, the explants were replenished with medium containing the same concentrations of sICAM-1. The highest titers observed determined the day of peak virus replication.

Virus neutralization and stabilization by sICAM-1. Infectivity reduction assays were performed as described previously (3) and were used to test whether sICAM-1 directly inactivates HRV-39 infectivity. HRV-39 (10^6 or 10^4 $TCID_{50}$s) was incubated in 1 ml of medium with or without 32 μg of sICAM-1 per ml at 33°C for 60 min on a rocker platform. The virus-drug mixtures were then diluted serially ($\log_{10}$), and the residual infectious virus titers were determined in monolayers of WI-38 cells. Guinea pig anti-HRV-39 neutralizing antibody and the compound pirodavir served as controls.

To test the effect of sICAM-1 on stabilization against heat and acid inactivation, HRV-39 (10^6 $TCID_{50}$s) was incubated for 60 min in 1 ml of medium with or without 32 μg of sICAM-1 per ml at 33°C on a rocker platform. The mixtures were diluted 1 to 10 in 0.1 M citrate buffer (pH 5.0) or in plain medium. Virus dilutions exposed to acid were incubated for 30 min at room temperature, and those in medium exposed to heat were incubated at 56°C for 10 min. The acidity was neutralized by adding sodium bicarbonate, and serial $\log_{10}$ dilutions were made for virus titration in WI-38 cell monolayers.

Effect of time of addition of sICAM-1 on yield of HRV-39 from a single round of replication. To examine the effect of sICAM-1 on the HRV replication cycle, experiments were done in which sICAM-1 was added at different times of a single cycle replication. Quadruplicate confluent monolayers of HeLa R19 cells in 96-well plates were treated with sICAM-1 at a final concentration of 10 μg/ml in maintenance medium beginning 1 h before or at different times (0, 15, 30, 60, or 180 min) after infection with HRV-39. To test for residual effects, one group of monolayers was exposed to sICAM-1 for 1 h and was then washed four times before virus inoculation. All monolayers were washed three times and were infected with HRV-39 diluted in maintenance medium at a multiplicity of infection of 3.0. After 60 min of incubation at 33°C, the monolayers were washed three times with phosphate-buffered saline (PBS) to remove unabsorbed virus and were then replenished with medium containing the same concentration of sICAM-1. For each time of addition of sICAM-1, a group of wells treated with plain maintenance medium was used as a control. Pirodavir (0.1 μg/ml) was also used as a control. At 10 h postinfection, the cultures were frozen and thawed three times, and the harvests that were clarified by centrifugation were titrated in WI-38 cell monolayers.

Effect of sICAM-1 on HRV-39 absorption or penetration in HeLa cells. To assess the effect of sICAM-1 on virus penetration, single-cycle replication experiments were conducted by using a modification of previously described procedures (11). Confluent HeLa cell monolayers were inoculated with HRV-39 at a multiplicity of infection of 0.3 in the presence or

TABLE 1. Effect of serotype and cell line on sICAM-1 inhibition of HRV CPE

HRV serotype	Cell line	EC_{50} (μg/ml)		Inhibition of CPE by pirodavir (0.1 μg/ml)[a]
		sICAM-1	R6.5	
3	WI-38	3.3	ND[b]	Yes
	HeLa	7.9	ND	Yes
5	WI-38	1.3	<0.1	Yes
14	WI-38	3.8	<0.1	Yes
	HeLa	>32	<0.1	Yes
16	WI-38	0.3	ND	Yes
	HeLa	2.6	ND	Yes
28	WI-38	3.0	<0.1	Yes
34	WI-38	1.6	ND	Yes
36	WI-38	0.1	<0.1	Yes
39	WI-38	0.4	0.6	Yes
	HeLa	1.8	<0.1	Yes
54	WI-38	1.9	>32	Yes
	HeLa	0.6	0.1	Yes
59	WI-38	1.0	<0.1	Yes
	HeLa	2.8	0.1	Yes
64	WI-38	3.0	<0.1	Yes
	HeLa	>32	<0.1	Yes
66	HeLa	6.8	<0.1	Yes
1A	WI38	>32	>32	Yes[c]
87	WI38	>32	>32	No

[a] Yes, complete inhibition of CPE; No, no inhibition of CPE.
[b] ND, not determined.
[c] Partial inhibition of CPE.

absence of sICAM-1 (10 μg/ml). Absorption was carried out for 1 h at 4°C; and the monolayers were washed three times with ice-cold medium, replenished with medium with or without sICAM-1, and incubated for 10 h at 33°C. The monolayers were frozen and thawed three times, and the virus titers were determined in WI-38 fibroblasts.

Additional experiments were done to assess the ability of anti-HRV-39 monoclonal antibodies to neutralize the remaining virus infectivity after the absorption period. Previously described assays (6, 11) were used, with modifications. After absorption of HRV-39 onto HeLa cells was carried out at 4°C in the presence or absence of sICAM-1 (10 μg/ml), monolayers were incubated at 33°C and neutralizing doses of antibody were added at different times (0, 30, 60, and 90 min) after absorption. After 10 h, the supernatants were removed; monolayers were washed two times with cold PBS, replenished with medium, and frozen and thawed three times; and the virus titers in WI-38 fibroblasts were determined.

RESULTS

Cytotoxicity of sICAM-1 to cell cultures. No morphological cytotoxic effects were observed on WI-38 or HeLa cells grown in the presence of sICAM-1 at concentrations of up to 100 μg/ml. By counting the number of viable cells in monolayers grown in the presence of various concentrations of sICAM-1 on the third and sixth days after seeding, we found that there was no inhibitory effect of sICAM-1 on the growth of WI-38 or HeLa cells.

Inhibitory activity of sICAM-1 for a range of rhinovirus serotypes. sICAM-1 was inhibitory for the replication of the 10 HRV serotypes of the major receptor group. For most of the HRV serotypes tested, the EC_{50}s reproducibly ranged between 0.1 and 7.9 μg/ml (Table 1). Serotypes HRV-14 and HRV-64 had EC_{50}s that were reproducibly higher in HeLa cells than they were in WI-38 cells, and HRV-64 was not inhibited by the highest sICAM-1 concentration used. sICAM-1 had no inhibitory effect on one serotype of the minor receptor group (HRV-1A) or on HRV-87, a serotype that has been demonstrated to belong to neither minor nor major receptor group (16). When the virus back-titration showed that inocula containing more than 32 to 256 $TCID_{50}$s had been used, higher EC_{50}s for sICAM-1 were consistently observed. The inhibitory concentrations of receptor-blocking antibody (R6.5) were lower on a weight basis than the concentrations of sICAM-1 for most but not all serotypes (Table 1). The capsid-binding agent pirodavir (0.1 μg/ml) was completely inhibitory for most of the serotypes tested; it was not inhibitory for HRV-87 but was partially inhibitory for HRV-1A.

TABLE 2. Inhibition of HRV-39 and HRV-59 replication in human respiratory epithelium[a]

HRV serotype	sICAM-1 concn (μg/ml)	No. of expts	% of expts with no replication	Peak virus titer (log_{10} $TCID_{50}$/ml)[b]
39	0	11	0	2.65 ± 1.04[c]
	1.0	5	0	1.83 ± 0.87
	3.2	6	33	1.46 ± 0.81[d]
	10.0	7	100[e]	≤0.5 ± 0.0[f]
59	10.0	2	100	≤0.5 ± 0.0

[a] Pirodavir (0.1 μg/ml) was completely inhibitory for HRV-39 and HRV-59 replication in two experiments.
[b] Values are means ± standard deviations.
[c] For the purpose of calculation, the absence of virus in the supernatant was expressed as 0.5 log_{10} $TCID_{50}$/ml.
[d] $P < 0.01$ versus no sICAM-1.
[e] $P < 0.05$.
[f] $P < 0.01$.

Effect of sICAM-1 on the yield of HRV-39 from multiple rounds of replication on human respiratory epithelium. As was expected from the results of a previous report (17), no detectable CPE was observed after inoculation of adenoid explant outgrowths with HRV-39. Virus replication was detectable in controls in all experiments, and the peak virus yields in control monolayers averaged 2.6 log_{10} $TCID_{50}$s/ml (Table 2). The peak virus yields in the presence of sICAM-1 were reduced in a concentration-dependent fashion (Table 2). sICAM-1 concentrations of 10 μg/ml were completely inhibitory in seven experiments. In contrast, sICAM-1 concentrations of 1 μg/ml did not reproducibly reduce virus yields compared with those in controls, and sICAM-1 at 3.2 μg/ml was completely inhibitory in one-third of the experiments, with an average virus titer reduction of 1.2 log_{10} $TCID_{50}$s/ml compared with that in controls. In other experiments, sICAM-1 at 10 μg/ml was inhibitory for a second major receptor group of rhinovirus (HRV-59) in the same system. Pirodavir (0.1 μg/ml) consistently inhibited virus growth.

Neutralization and stabilization of HRV-39 by sICAM-1. No reduction of HRV-39 infectivity was observed following incubation with 32 μg of sICAM-1 per ml for 1 h at 33°C (Fig. 1). When the incubation period was extended to 24 h, HRV-39 infectivity was reduced by only 0.25 log_{10} $TCID_{50}$/ml. Under the same conditions, the antipicornavirus capsid-binding agent pirodavir reduced viral infectivity by approximately 1 log_{10} $TCID_{50}$, and neutralizing polyclonal antibody was associated with a greater than 4-log_{10}-$TCID_{50}$ reduction. Also, sICAM-1 did not exert any protective effect against virus inactivation by heat or acid (data not shown).

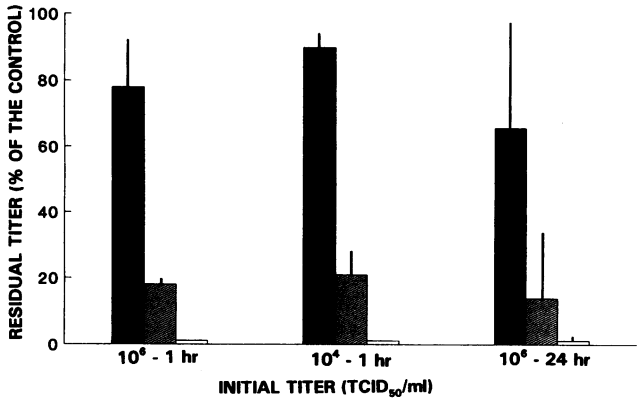

FIG. 1. Effect of incubation with sICAM-1 (32 μg/ml) on HRV-39 infectivity. Residual virus titers after incubation for 1 or 24 h are expressed as a percentage of the titer of a control incubated with plain medium. Pirodavir (0.1 μg/ml) and anti-HRV-39 monoclonal antibody were used as positive controls. The lines above the bars represent one standard deviation of the mean of four experiments. Solid bars, sICAM-1; hatched bars, pirodavir; open bars, anti-HRV-39 monoclonal antibody.

Effect of sICAM-1 on the yield of HRV-39 from single round of replication. Following a single round of replication, the yields of virus in control HeLa monolayers averaged 5.0 $\log_{10}$ $TCID_{50}$s/ml (range, 4.75 to 6.25 $\log_{10}$ $TCID_{50}$s/ml). In comparison with controls, virus yields were reduced when 10 μg of sICAM-1 per ml was added 1 h before infection, at the time of infection, or shortly after infection (Fig. 2). At 30 min, less than 1.0 $\log_{10}$ reduction in yield was observed, and after 1 h sICAM-1 had no inhibitory effect on the yield of HRV-39. When pirodavir, which was used as a positive control, was added, there was a reduction in virus yield greater than or equal to 1.0 $\log_{10}$ $TCID_{50}$/ml when it was added 30 min after infection, but there was only a 0.25 $\log_{10}$ $TCID_{50}$/ml reduction at 60 min and no reduction at 180 min.

When 32 μg of sICAM-1 per ml or 0.2 μg of control pirodavir per ml was added to monolayers for 1 h and then removed by washing them three times with PBS and inoculated with HRV-39 in the absence of sICAM-1, no reduction in virus yield was observed in comparison with that in untreated control monolayers (data not shown).

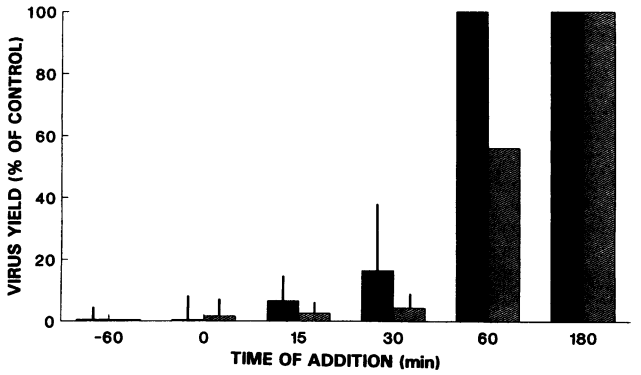

FIG. 2. Effect of timing of addition of sICAM-1 (10 μg/ml) on the yield of single round of replication of HRV-39 in HeLa cells. Virus yields are expressed as a percentage of the yield of controls (cultures treated with no drug). Pirodavir (0.1 μg/ml) was used as positive control. The lines above the bars represent one standard deviation of the mean of three experiments. Solid bars, sICAM-1 (10 μg/ml); hatched bars, pirodavir (0.1 μg/ml).

TABLE 3. Effect of sICAM-1 on attachment of HRV-39 to HeLa cells

sICAM-1 (10 μg/ml)		Virus yield ($\log_{10}$ $TCID_{50}$/ml)[a]
Absorption (1 h, 4°C)	Incubation (10 h, 33°C)	
Absent	Absent	5.4
Absent	Present	5.0
Present	Absent	3.0
Present	Present	3.0

[a] Values are means of two experiments in which quadriplicate wells were used for each condition.

Effect of sICAM-1 on the absorption of HRV-39 to HeLa cells. In controls, when sICAM-1 was absent during the absorption and subsequent incubation periods, virus titers peaked at 5.4 $\log_{10}$ $TCID_{50}$s/ml. Virus titer reductions of 2 $\log_{10}$ $TCID_{50}$s were observed only when sICAM-1 was present during the absorption period, regardless of its presence or absence during the subsequent postabsorption incubation period. When sICAM-1 was absent during the absorption period and present only during the postabsorption incubation period, no virus titer reduction was observed (Table 3). That observation was further confirmed by the results that we obtained in experiments in which we tested antibody-mediated neutralization of infectivity. As expected, the titer of virus that was already internalized in the cell, and that was therefore nonneutralizable, increased with the delayed addition of neutralizing antibody to the cultures that were not treated with sICAM-1. However, the virus titer remained practically constant in the sICAM-1-treated cultures (Table 4), a result that is consistent with an effect of sICAM-1 in the early steps of the infection cycle, during attachment or internalization.

DISCUSSION

We found that sICAM-1 is inhibitory to the replication of 10 serotypes of the major HRV receptor group in at least one cell line. The EC_{50}s observed for most of the serotypes tested (0.1 to 7.9 μg/ml) are consistent with those obtained for the infectivity of HRV-54 by using the same sICAM-1 preparation (10) and were about 1 order of magnitude lower than those obtained for the infectivity of HRV-3 by using another preparation of soluble ICAM-1 in HeLa cells (8).

TABLE 4. Effect of sICAM-1 on HRV-39 penetration in HeLa cells[a]

Time (min) of addition of neutralizing antibody	Residual viral infectivity ($\log_{10}$ $TCID_{50}$/ml)[b]	
	sICAM-1 (10 μg/ml)	Control
0	1.5	2.0
30	1.25	2.5
60	1.5	3.0
90	1.25	3.0

[a] The effect was determined by measuring the residual infectivity after neutralization of noninternalized virus with anti-HRV-39 monoclonal antibody at different times.

[b] Values are the means of two experiments in which quadruplicate wells were used for each time of addition of antibody.

Use of a 24-h incubation, which is longer than one cycle of HRV replication, may have been the cause of the relatively higher EC_{50}s observed by those investigators (8). As expected, sICAM-1 was not inhibitory for serotypes HRV-1A, which is a member of the minor receptor group, or HRV-87, which has been demonstrated not to use ICAM-1 as a receptor (16).

The inhibitory concentrations of sICAM-1 varied for the major receptor group serotypes tested and appeared to be cell type dependent. For example, serotype HRV-64-infected HeLa cells were completely insensitive to CPE inhibition by the highest sICAM-1 concentration tested. One of the reasons for the cell type-dependent differences in sensitivity to sICAM-1 inhibition is the variable susceptibilities of the cell lines to infection by different serotypes of HRV as a result of quantitative or qualitative differences in ICAM-1 expression. In addition, some serotype-related variation in sensitivity to the antiviral effect of sICAM-1 could be related to small differences in the shape of the canyon wall and/or differences in virus receptor affinity between serotypes (2). These cell type- and serotype-dependent variabilities in activity may be evidence that the inhibitory effect of sICAM-1 on HRV infection is not simply a direct consequence of the blockade of the receptor attachment site of the virus. One possibility is that the steric interferences created by interactions between less distal domains of sICAM-1 and the residues present on the walls or on the edge of the canyons of certain serotypes may be of variable importance for the antirhinoviral effect of sICAM-1 (5). The variability in susceptibility to sICAM-1 inhibition among the serotypes in different cell lines observed in this study indicates that a broader range of viruses of the major receptor group needs to be tested and that additional mechanism of action studies with different serotype-cell combinations are necessary for a better understanding of this issue.

We also found that sICAM-1 inhibited the replication of a representative major receptor group serotype in primary cultures of human adenoid epithelium in a concentration-dependent manner. Although it is subject to variability because of tissue manipulation and host-related variations in susceptibility to HRV infection, the system has been shown to support HRV replication reproducibly and has been shown to be useful for the evaluation of antiviral activity in the natural HRV host cell (3). The inhibitory concentrations observed for HRV-39 in this system were comparable to those observed in HeLa or WI-38 cells.

The observed lack of a significant reduction in the infectivity of HRV-39 by direct incubation of HRV-39 with excess sICAM-1 and the failure of sICAM-1 to protect HRV against inactivation by acid or heat indicate that the virus–sICAM-1 interaction is readily reversible. This observation has been documented recently for HRV-3 (8). The same group of investigators (8) reported that, after 24 h of incubation, the interaction of sICAM-1 with HRV can result in uncoating of the virus and irreversible conversion of 60% of the virions to an altered form that sediments at 80 S and that lacks the VP4 protein and RNA. The insignificant reduction of infectivity that we observed after up to 24 h of incubation suggests that a conversion to empty virions does not seem to be a major mechanism for the neutralization of HRV infectivity by sICAM-1. It remains to be determined whether a multimeric form of the molecule would achieve direct inactivation of virus infectivity.

Time-of-addition studies showed that a reduction in the HRV-39 yield was observed only when sICAM-1 was present during the absorption period. This indicates that the inhibitory effect of sICAM-1 is present early in the infection cycle, probably during attachment. This early inhibitory effect was also observed for HRV-39 in experiments on human adenoid explants, in which the addition of sICAM-1 after virus absorption failed to decrease virus yields. Moreover, the increase in internalized virus titer in untreated cultures in which neutralizing antibody addition was delayed and the almost constant virus yields in the sICAM-1-treated cultures are consistent with inhibition of virus attachment or internalization by sICAM-1. This mechanism of action has been suggested to involve domains 3, 4, and 5 of sICAM-1 (8). Competition of sICAM-1 for receptor-binding sites on the virus has been shown to occur (10) and has been suggested as one of the mechanisms of the antirhinovirus effect of sICAM-1 (8). Our studies did not attempt to study competitive binding directly, but our results are consistent with that mechanism.

In conclusion, we showed that sICAM-1 inhibits the replication of 10 major group receptor serotypes of HRV in human embryonic lung fibroblasts and HeLa cells in a serotype- and time-dependent manner and also inhibits HRV-39 replication in vitro in human respiratory epithelium in a concentration-dependent manner. While the interaction of sICAM-1 with HRV seems to be readily reversible by dilution, the antirhinoviral action of sICAM-1 seems to occur during the early steps of the infection cycle, probably by blocking attachment or entry. The serotype-related differences in sensitivity to the CPE inhibition effect of sICAM-1, which were observed in particular in HeLa cells, warrant susceptibility testing of a broader range of serotypes. The rapid reversibility of the sICAM-1–HRV interaction suggests that sICAM-1 would need to be maintained in the respiratory secretions in order to exert a maximal antiviral effect.

ACKNOWLEDGMENTS

This work was supported in part by a gift from Boehringer Ingelheim Pharmaceuticals Inc.

We thank Austin Sydnor, Jr., and associates for providing the human adenoids, Koen Andries for supplying pirodavir and for helpful suggestions, and Nancy Knott for secretarial support.

REFERENCES

1. **Abraham, G., and R. J. Colonno.** 1984. Many rhinovirus serotypes share the same cellular receptor. Proc. Natl. Acad. Sci. USA **51**:340–345.
2. **Abraham, G., and R. J. Colonno.** 1988. Characterization of human rhinoviruses displaced by an anti-receptor monoclonal antibody. J. Virol. **62**:2300–2306.
3. **Andries, K., B. Dewindt, M. De Brabander, R. Stokbroekx, and P. A. J. Janssen.** 1988. In vitro activity of R 61837, a new antirhinovirus compound. Arch. Virol. **101**:155–167.
4. **Andries, K., B. Dewindt, J. Snoeks, R. Willebrords, K. Van Eemeren, R. Stokbroekx, and P. A. J. Janssen.** 1992. In vitro activity of pirodavir (R 77975), a substituted phenoxy-pyridazinamine with broad-spectrum antipicornaviral activity. Antimicrob. Agents Chemother. **36**:100–107.
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 rhinovirus canyon in receptor binding. Proc. Natl. Acad. Sci. USA **85**:5449–5453.
6. **Eggers, H. J.** 1977. Selective inhibition of uncoating of echovirus 12 by rhodanine. A study on early virus-cell interaction. Virology **78**:241–252.
7. **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.
8. **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.

9. **Marlin, S. D., and T. A. Springer.** 1987. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen (LFA-1). Cell **51**:813–819.
10. **Marlin, S. D., D. E. Staunton, T. A. Springer, C. Stratowa, W. Sommergruber, and V. J. Merluzzi.** 1990. A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection. Nature (London) **344**:70–72.
11. **McSharry, J. J., L. A. Caliguiri, and H. J. Eggers.** 1979. Inhibition of uncoating of poliovirus by arildone, a new antiviral drug. Virology **97**:307–315.
12. **Rothlein, R., M. Czajkowski, M. M. O'Neil, S. D. Marlin, E. Mainolfi, and V. J. Merluzzi.** 1988. Induction of intercellular adhesion molecule 1 on primary and continuous cell lines by pro-inflammatory cytokines. Regulation by pharmacologic agents and neutralizing antibodies. J. Immunol. **141**:1665–1669.
13. **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.
14. **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.
15. **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.
16. **Uncapher, C. R., C. M. DeWitt, and R. J. Colonno.** 1991. The major and minor group receptor families contain all but one human rhinovirus serotype. Virology **180**:814–817.
17. **Winther, B., J. M. Gwaltney, Jr., and J. O. Hendley.** 1990. Respiratory virus infection of monolayer cultures of human nasal epithelial cells. Am. Rev. Respir. Dis. **141**:839–845.