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Comparative Antirhinoviral Activities of Soluble Intercellular Adhesion Molecule-1 (sICAM-1) and Chimeric ICAM-1/Immunoglobulin A Molecule

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We conducted a comparative study of the antirhinovirus activities of soluble intercellular adhesion molecule-1 (sICAM-1) and a chimeric ICAM-1/immunoglobulin A (IgA) molecule (IC1-5D/IgA) for nine major receptor group human rhinovirus (HRV) serotypes and for a variant of HRV-39 relatively resistant to inhibition by sICAM-1. IC1-5D/IgA inhibited the infectivity of eight of the nine wild-type HRVs and the resistant HRV-39 variant and was 60 to 170 times more potent than sICAM-1 on a molar basis. In contrast to sICAM-1, IC1-5D/IgA directly neutralized the infectivity of the representative HRVs by ~1 $\log_{10}$. These results expand on the antirhinovirus spectrum of IC1-5D/IgA, confirm that dimeric forms of sICAM-1 have a higher antirhinoviral potency than monomeric sICAM-1, and indicate that cross-linking of two adjacent receptor binding sites on the virus capsid by a divalent receptor enhances the direct inactivation of viral infectivity.

Intercellular adhesion molecule-1 (ICAM-1) has been identified as the cell receptor for the major group of human rhinoviruses (HRVs) (4, 9, 10). Major HRVs bind to the N-terminal immunoglobulin-like domain (D1) of ICAM-1 through residues located in the central portions of the floor of the canyon that surrounds the fivefold vertex of the virus capsid (8). Recombinant soluble forms of ICAM-1 (sICAM-1) have antirhinoviral activity in vitro, which is mediated through three characterized mechanisms of action: competition for the receptor-binding sites on the virus, hindrance of an early infection event such as entry or uncoating, and, to a substantially lesser extent, direct inactivation with formation of empty capsids (2, 5–7).

One such sICAM-1 (6) was shown to have antirhinoviral activity in vitro against 88 of the 90 numbered HRVs belonging to the major receptor group, with 50% effective inhibitory concentrations (EC_{50}) ranging from 0.1 to 41.1 μg/ml in WI-38 human embryonic lung fibroblast cells (3). Chimeric immunoadhesin molecules have been constructed from either the two most distal domains of ICAM-1 coupled with the heavy chain of immunoglobulin A1 (IgA1), IgG, or IgM, or from all five extracellular domains of ICAM-1 coupled with the heavy chains of IgA1 or IgG (7). The most active of these molecules, a chimera composed of the five extracellular domains of ICAM-1 coupled with the heavy chain of IgA (IC1-5D/IgA), was shown by plaque reduction assay to inhibit the infectivity of HRV-3 approximately 200-fold more effectively on a molar basis than monomeric sICAM-1. IC1-5D/IgA also inhibited the binding of HRV-3 to HeLa cells approximately 7-fold more effectively than monomeric sICAM-1 and was approximately 12-fold more efficient than sICAM-1 in inducing formation of empty capsids of HRV-3 (7).

We have done a comparative study of the inhibitory activities of sICAM-1 (provided by Steven D. Marlin, Boheringer Ingelheim Pharmaceuticals, Ridgefield, Conn.) and the chimera IC1-5D/IgA (provided by Timothy A. Springer, Harvard Medical School, Boston, Mass.) for nine major HRV serotypes (serotypes 3, 13, 14, 16, 23, 39, 68, 73, and 80) and for a variant of HRV-39 selected for moderate resistance to sICAM-1 (1). All of the wild-type major HRV serotypes were originally obtained from the American Type Culture Collection. The EC_{50}s were determined by cytopathic effect inhibition assay in WI-38 cells as described previously (2). The chimera IC1-5D/IgA inhibited the infectivity of eight wild-type major HRV serotypes tested with EC_{50}s between 0.02 and 0.48 μg/ml (equivalent to binding site concentrations between 0.2 and 4.8 nM) (Table 1). IC1-5D/IgA was more potent than monomeric sICAM-1 by 50 to 143 times on a weight basis and by 60 to 170 times on a molar basis. These results are consistent with and expand on the previously reported inhibition of one HRV serotype by IC1-5D/IgA (7). Moreover, the more potent activity of the divalent IC1-5D/IgA that we observed against multiple HRV serotypes compared with that of the monomeric sICAM-1 is in keeping with the hypothesis that multivalent binding results in higher virus-receptor affinity (7).

We have previously isolated a variant of HRV-39 moderately resistant to sICAM-1 by serial passages in HeLa cells in the presence of 100 μg of sICAM-1 per ml, a concentration ~100 times the EC_{50} for the wild-type virus (1). The EC_{50} value for this variant was ~30-fold higher than the value for the wild-type virus, an increase that is within the ~400-fold range of values reported for the numbered major HRV serotypes (3). Direct and indirect evidence suggested that the sICAM-1 resistance was a preexisting phenotype selected from the pool of wild-type virus presumably because of an altered binding phenotype with reduced virus-receptor affinity (1). In the present study, the moderately resistant variant designated HRV-39/7p6 had a sICAM-1 EC_{50} value 38-fold higher than that of the wild-type HRV-39 (Table 1). In contrast, the EC_{50} value of IC1-5D/IgA was only modestly (approximately fivefold) increased for the sICAM-1-resistant variant compared with the wild-type HRV-39 (Table 1). This observation may suggest that the reduction in receptor-binding affinity of the resistant variant, which may result from a slight canyon conformational variation(s), can be partially compensated by the

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TABLE 1. Inhibitory effect of sICAM-1 and chimeric ICAM-1/IgA molecule (IC1-5D/IgA) on HRV cytopathic effect in human embryonic lung fibroblasts (WI-38 strain)

HRV serotype	EC_{50} (μg/ml)[a]		Ratio of sICAM-1 EC_{50} to IC1-5D/IgA EC_{50}
	sICAM-1	IC1-5D/IgA	
3	5.7 ± 0.6	0.04 ± 0.01	143
13	20.6 ± 7.1	0.27 ± 0.12	76
14	6.8 ± 0.8	0.06 ± 0.01	113
16	1.2 ± 0.1	0.02 ± 0.01	60
23	>100[b]	>32.0	
39	1.0 ± 0.3	0.02 ± 0.01	50
39/7p6[c]	38.8 ± 22.6	0.10 ± 0.09	388
68	7.9 ± 2.3	0.07 ± 0.04	113
73	30.4 ± 4.9	0.48 ± 0.47	63
80	52.1 ± 27.1	0.42 ± 0.40	124

[a] Values are means ± standard deviations from two to three independent assays.
[b] Result taken from previously published assays (3).
[c] HRV-39 variant moderately resistant to inhibition by sICAM-1 (1).

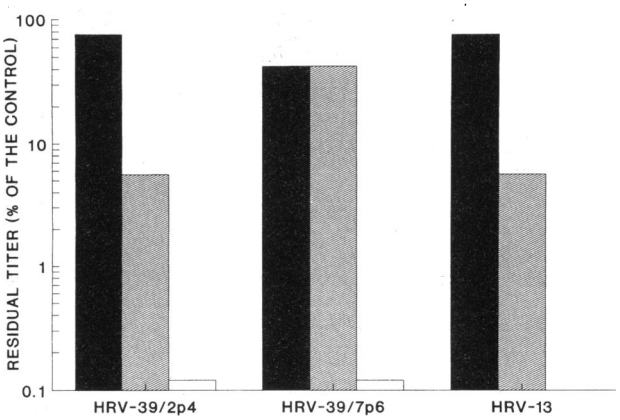

FIG. 1. Effect of incubation with sICAM-1 or IC1-5D/IgA on the infectivity of plaque purified wild-type HRV-39/2p4, HRV-39/7p6 (a variant of HRV-39 selected for resistance to sICAM-1), and pooled culture supernatant of HRV-13. Residual virus titers obtained after 1 h of incubation of 10^6 50% tissue culture infective doses with concentrations equal to 10 times the EC_{50} value of each molecule for the respective virus are expressed as percentages of the titer of a control incubated with plain medium. Concentrations of sICAM-1 were 10 μg/ml for HRV-39/2p4, 390 μg/ml for HRV-39/7p6, and 210 μg/ml for HRV-13; concentrations of IC1-5D/IgA were 0.2 μg/ml for HRV-39/2p4, 1 μg/ml for HRV-39/7p6, and 3 μg/ml for HRV-13. IC_{50} values are the average of two independent experiments. Symbols: solid bars, sICAM-1; hatched bars, IC1-5D/IgA; open bars, anti-HRV-39 guinea pig neutralizing antibody used as a positive control.

higher virus-receptor affinity consequent to multivalency. Martin and colleagues postulated that virus escape from inhibition by multivalent immunoadhesins would be expected to occur at a lower frequency than that to monomeric soluble receptor (7). Our data indicate that the IC1-5D/IgA molecule retains a greater relative inhibitory effect for a virus selected for relative resistance to sICAM-1 and is consistent with this hypothesis.

In a previous study (3), we found that HRV serotypes 23 and 25, previously classified as belonging to the major receptor group (11), were not susceptible to inhibition by 100 μg of sICAM-1 per ml (~1.2 μM). In addition, receptor specificity studies using receptor blocking with an excess of anti-ICAM-1 monoclonal antibody in two cell lines suggested that those two serotypes used a cell receptor different from ICAM-1 (3). In the present study, IC1-5D/IgA also had no effect at the highest concentration tested on the infectivity of HRV-23 (Table 1). This suggests that the valency of the soluble receptor does not alter the susceptibility of HRV-23 and provides additional evidence that HRV-23 may use a different cell receptor.

We have previously shown by infectivity reduction assays that HRV-39 was not directly inactivated to a significant extent (<0.5 $\log_{10}$ reduction in infectivity) by incubation with monomeric sICAM-1 for up to 24 h (2). In the present study, we assessed the neutralizing effect of IC1-5D/IgA on the infectivity of two major HRV serotypes, HRV-39 and HRV-13, and the variant of HRV-39 resistant to sICAM-1, as described previously (2). Briefly, approximately 10^6 50% tissue culture infective doses of each virus were incubated in medium containing a concentration of sICAM-1 or IC1-5D/IgA equal to ~10 times the IC_{50} of each molecule for the respective virus, or in plain medium, for 1 h at 33°C on a rocker platform. Each virus-drug or virus-medium mixture was then serially diluted in 10-fold dilutions, and the infectivity was determined on quadruplicate monolayers of WI-38 cells in 96-well plates. Guinea pig neutralizing antibody to HRV-39 (American Type Culture Collection) was used as a positive control for the HRV-39 assays. Confirming previous observations, there was no significant reduction of infectivity of HRV-39, HRV-13, or HRV-39/7p6 after incubation with sICAM-1 (Fig. 1). A reduction in infectivity of HRV-39 and HRV-13 of approximately 1.0 $\log_{10}$ was observed after incubation with IC1-5D/IgA. This observation is in keeping with data previously published by Martin et al. (7), who found that IC1-5D/IgA was roughly 12 times more efficient that sICAM-1 in inducing conformational changes in the virus capsid. In contrast to wild-type HRV-39, no significant reduction in the infectivity of HRV-39/7p6 was observed after incubation with IC1-5D/IgA (Fig. 1). This result indicates that cross-linking adjacent receptor binding sites by means of multivalency of the soluble receptor does not affect its ability to directly inactivate a HRV-39 variant that binds the receptor with lower affinity.

The enhanced antirhinoviral potency of this multimeric molecule of sICAM-1 and its direct inactivation of some HRV serotypes make it an interesting one for further study and potential clinical development.

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