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Echovirus 1 Interaction with the Isolated VLA-2 I Domain

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The isolated I domain of the integrin VLA-2, produced as a bacterial fusion protein, specifically bound echovirus 1 and prevented virus attachment to cells. These results demonstrate that the receptor structures critical for virus attachment are contained solely within the VLA-2 I domain and that soluble receptor fragments are capable of preventing infection.

Echovirus 1 initiates infection by attaching to the integrin VLA-2 (CD49b/CD29) (2), a cell surface glycoprotein that mediates cell interactions with collagen and laminin (8). VLA-2 is a heterodimer composed of a 150-kDa α2 subunit associated with the 130-kDa β1 subunit common to all VLA proteins (8). Ligand specificity, including the capacity to bind virus, depends on the α2 subunit (3, 8). A prominent feature of the α2 protein is the I ("inserted") domain (16), a 200-amino-acid region similar to the A domains of von Willebrand's factor, cartilage matrix protein, and complement proteins and to domains (termed either I or A by different authors) present in the β2 leukocyte integrins (6). Experiments using interspecies chimeras and epitope mapping with function-blocking monoclonal antibodies have identified the I domain as critical for VLA-2 interactions with both virus (4) and collagen (4, 9), suggesting that the ligand binding sites are contained within this portion of the molecule. However, those experiments did not demonstrate a direct interaction between virus and the I domain and could not exclude the possibility that other regions within the α2 or β1 subunits were also essential components of the virus binding site. We therefore determined whether echovirus 1 could bind directly to the isolated VLA-2 I domain synthesized as a bacterial fusion protein.

Specific virus binding to the human I domain-GST fusion protein. DNA encoding the I domain (amino acids 140 to 339) was amplified from a human α2 cDNA clone (16), using PCR primers that introduced *Bam*HI and *Eco*RI restriction sites at the ends of the 650-bp fragment to permit unidirectional insertion into the pGEX-KT glutathione-S-transferase (GST) expression vector (7). The human I domain–GST fusion protein was produced in bacteria and purified with glutathione-Sepharose essentially as described previously (7) (Fig. 1). GST with no inserted domain was similarly prepared, as was a GST fusion protein incorporating the A domain of the human leukocyte integrin CR3 (CD11b/CD18) (from bacterial extracts provided by M. Amin Arnaout [12]).

To determine whether echovirus 1 bound to the I domain fusion protein, [^{35}S]methionine-labeled virus (prepared as described previously [2]) was incubated for 1 h at room temperature with purified fusion proteins immobilized on glutathione-Sepharose beads (Fig. 2). Beads were then washed to remove unbound virus and dissolved for scintillation counting. Echovirus 1 bound specifically to the I domain fusion protein but did not bind to GST protein with no inserted domain or to the CR3 A domain fusion protein. A control virus, coxsackievirus B3, did not bind to any of the fusion proteins. These results

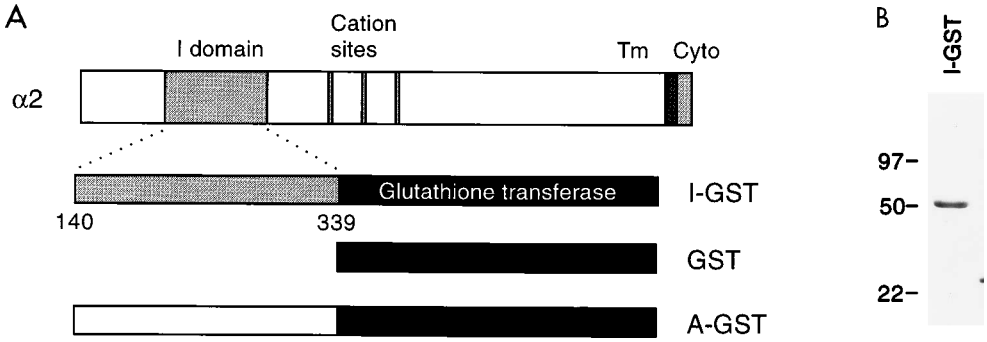

FIG. 1. GST fusion proteins. (A) Structure of the VLA-2 α2 subunit, showing the I domain, three divalent cation binding sites believed to regulate cation-dependent cell interactions with collagen and laminin, and the transmembrane (Tm) and cytoplasmic (Cyto) domains. DNA encoding the VLA-2 I domain (amino acids 140 to 339 as defined in reference 16) was inserted into the pGEX-KT vector, the nucleotide sequence was confirmed, and a fusion protein incorporating the I domain at the N terminus of the *Schistosoma japonicum* GST was isolated from transformed bacteria by chromatography on glutathione-Sepharose. Control proteins, GST with no added protein domain and a GST fusion protein incorporating the homologous A domain of the leukocyte integrin CR3 (A-GST), were prepared in the same way. (B) Purified fusion proteins subjected to electrophoresis in a 12% polyacrylamide gel. The positions of molecular size standards (in kilodaltons) are indicated on the left. I-GST, human VLA-2 I domain–GST fusion protein; GST, GST protein; A-GST, CR3 A domain–GST fusion protein.

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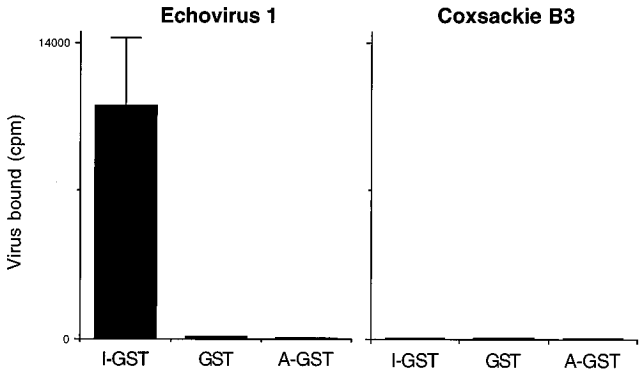

FIG. 2. Specific virus binding to the human I domain–GST fusion protein. The I-domain fusion protein (I-GST), GST protein (GST), and CR3 A domain–GST fusion protein (A-GST) were immobilized on glutathione-Sepharose beads. Aliquots containing 15 μg of bound protein were incubated with radiolabeled echovirus 1 (37,000 cpm) or coxsackievirus B3 (25,000 cpm) for 1 h in virus binding buffer as defined elsewhere (1). Beads were then washed to remove unbound virus and dissolved for scintillation counting. Results are shown as mean virus bound (in counts per minute) ± standard deviation for triplicate samples.

demonstrate that the structures required for binding echovirus 1 are contained within the VLA-2 I domain.

To test the specificity of echovirus 1 binding to the I domain fusion protein, we used anti-α2 monoclonal antibodies (MAbs) known to recognize distinct functional epitopes within the I domain (4). 5E8 blocks VLA-2 interactions with both virus and collagen; 12F1 prevents virus attachment but has no effect on VLA-2 adhesion to collagen; and Gi9 abrogates adhesion to collagen but has little effect on virus binding. MAb AA10, which also recognizes an epitope within the I domain, blocks virus attachment and has variable effects on collagen adhesion (1, 4, 5). Consistent with their effects on virus attachment to VLA-2 on HeLa cells (Fig. 3A), 5E8, 12F1, and AA10 markedly inhibited virus attachment to the I domain fusion protein, whereas Gi9 did not (Fig. 3B). MAb HAS-4, which recognizes an α2 epitope outside the I domain (4), had no inhibitory effect. These results indicate that virus attachment to the isolated I domain involves the same epitopes that are critical for virus interaction with intact VLA-2 and suggest that the structure of the VLA-2 I domain is preserved within the fusion protein.

Inhibition of echovirus 1 attachment and infection by soluble I domain fusion protein. HeLa cell monolayers were incubated with radiolabeled virus in the presence of different amounts of I domain fusion protein or control proteins (Fig. 4). The I domain fusion protein blocked 50% of virus attachment at approximately 1.5 μg/ml (30 nM), whereas the CR3 A domain and GST control proteins had no effect even at 75 μg/ml. In some experiments performed with different virus and protein preparations, inhibition was seen at 10-fold lower concentrations of fusion protein.

The I domain fusion protein also prevented echovirus infection, as determined by inhibition of plaque formation (Fig. 5). HeLa cell monolayers were incubated with echovirus 1 and either I domain or control proteins (15 μg/ml) for 1 h at room temperature; monolayers were then overlaid with agar and plaques were developed and visualized as described previously (2). The I domain fusion protein inhibited plaque formation by 94%, whereas control proteins had no inhibitory effect.

Discussion. These results demonstrate that the essential elements for echovirus 1 binding are contained within the I domain of the human integrin α2 subunit. As indicated by the MAb blocking experiments, echovirus interaction with the isolated I domain involves the same receptor structures that function in virus attachment to the intact cell surface molecule.

Inhibition experiments suggest that the interaction between a single I domain and its virus attachment site is sufficiently strong to compete with the potentially multivalent interactions between a virion and multiple receptor molecules on the cell surface. In different experiments, we observed that 3 to 30 nM I domain fusion protein blocked 50% of echovirus attachment to HeLa cells. In similar experiments with another picornavirus, other investigators found that 45 nM soluble intercellular adhesion molecule 1 (ICAM-1) blocks rhinovirus attachment by 50% (11). In a different viral system, the affinity of soluble human immunodeficiency virus gp120 for the CD4 receptor, determined in equilibrium binding studies, ranged between 1 and 20 nM for different viral strains (13).

Echovirus 1 and collagen interact with overlapping but nonidentical sites within the VLA-2 I domain, and binding of these

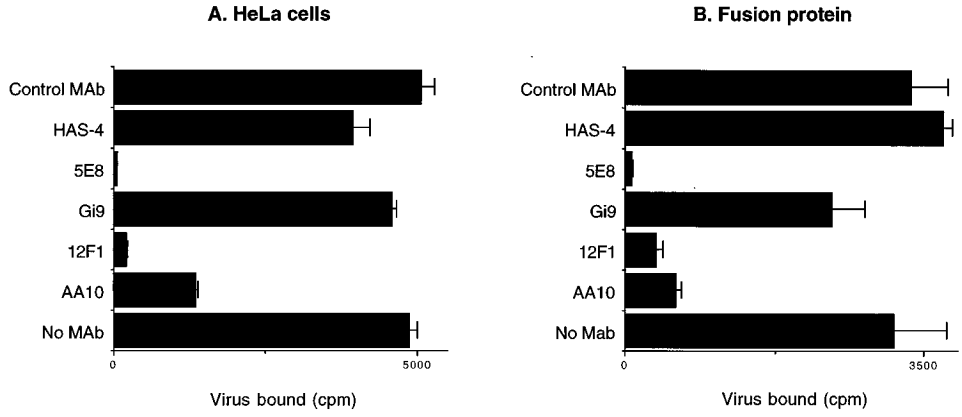

FIG. 3. Specific inhibition of echovirus 1 binding by anti-α2 MAbs. (A) Virus attachment to VLA-2 on HeLa cells. HeLa cell monolayers in 24-well tissue culture plates were incubated with MAbs (approximately 30 μg/ml). MAbs 5E8, 12F1, Gi9, and HAS-4 (obtained from the 5th International Conference on Human Leukocyte Differentiation Antigens) and AA10 (2) were previously mapped with respect to the I domain (4). An irrelevant immunoglobulin M control MAb which recognizes the B-lymphocyte-specific protein B7 was provided by Gordon Freeman. Washes and incubations with radiolabeled echovirus 1 (28,000 cpm) were performed as described previously (1). (B) Virus attachment to I domain fusion protein on glutathione-Sepharose beads. Aliquots containing 2 μg of protein were preincubated with MAbs, washed, and exposed to radiolabeled echovirus 1 (6,000 cpm) as described above for HeLa cells. Lower amounts of protein were used than in Fig. 2 to ensure antibody excess. Results are shown as mean virus bound (in counts per minute) ± standard deviation for triplicate samples.

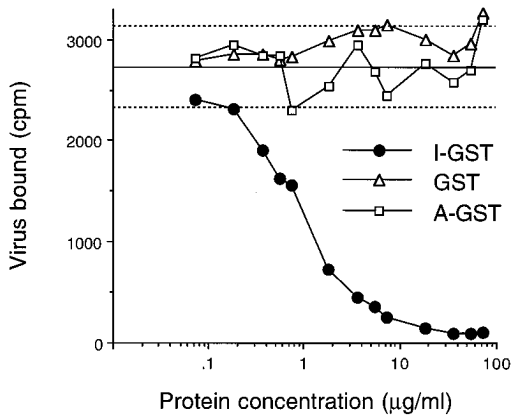

FIG. 4. Inhibition of virus attachment by the human I domain–GST fusion protein. HeLa cell monolayers in 24-well plates were incubated with radiolabeled echovirus 1 (14,000 cpm) in the presence of different amounts of I domain–GST fusion protein (I-GST), GST protein (GST), or CR3 A domain–GST fusion protein (A-GST). Results are shown as mean counts per minute bound to duplicate monolayers. Virus attachment to six control monolayers (incubated with virus in buffer alone) is also shown. The mean is indicated by the solid line, and the standard deviation is indicated by the broken lines.

two ligands is regulated independently (1, 4). The results shown here demonstrate that virus interacts directly with the I domain. While this work was in progress, Kamata and Takada (10) reported that the VLA-2 I domain, synthesized as a bacterial fusion protein, binds collagen, consistent with other recent reports of ligand interactions with integrin I domains (14, 15, 17, 18). The isolated VLA-2 I domain thus contains the binding sites both for virus and collagen, suggesting that its folding and overall structure are not significantly different from those of the I domain within the native $\alpha 2\beta 1$ heterodimer. Because integrin molecules like VLA-2 are beyond the reach of available crystallographic techniques, the isolated I domain described here may prove valuable for structural studies of virus-receptor interactions.

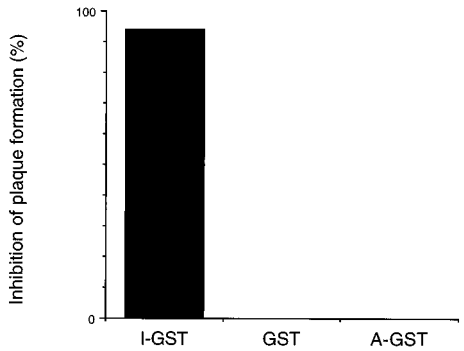

FIG. 5. Inhibition of plaque formation by the human I domain–GST fusion protein. HeLa cell monolayers in six-well tissue culture plates were incubated with echovirus 1 in medium alone or in medium containing 15 μg of human I domain–GST fusion protein (I-GST), GST protein (GST), or CR3 A domain–GST fusion protein (A-GST) per ml for 1 h at room temperature. Plaque assay conditions and calculation of percent inhibition are described in reference 3. Results are from duplicate assays.

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