Foot-and-Mouth Disease Virus Receptors: Comparison of Bovine α_V Integrin Utilization by Type A and O Viruses

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Three members of the α_V integrin family of cellular receptors, $\alpha_V\beta_1$, $\alpha_V\beta_3$, and $\alpha_V\beta_6$, have been identified as receptors for foot-and-mouth disease virus (FMDV) in vitro. The virus interacts with these receptors via a highly conserved arginine-glycine-aspartic acid (RGD) amino acid sequence motif located within the βG-βH (G-H) loop of VP1. Other α_V integrins, as well as several other integrins, recognize and bind to RGD motifs on their natural ligands and also may be candidate receptors for FMDV. To analyze the roles of the α_V integrins from a susceptible species as viral receptors, we molecularly cloned the bovine β_1, β_5, and β_6 integrin subunits. Using these subunits, along with previously cloned bovine α_V and β_3 subunits, in a transient expression assay system, we compared the efficiencies of infection mediated by $\alpha_V\beta_1$, $\alpha_V\beta_3$, $\alpha_V\beta_5$, and $\alpha_V\beta_6$ among three strains of FMDV serotype A and two strains of serotype O. While all the viruses could infect cells expressing these integrins, they exhibited different efficiencies of integrin utilization. All the type A viruses used $\alpha_V\beta_3$ and $\alpha_V\beta_6$ with relatively high efficiency, while only one virus utilized $\alpha_V\beta_1$ with moderate efficiency. In contrast, both type O viruses utilized $\alpha_V\beta_6$ and $\alpha_V\beta_1$ with higher efficiency than $\alpha_V\beta_3$. Only low levels of viral replication were detected in $\alpha_V\beta_5$-expressing cells infected with either serotype. Experiments in which the ligand-binding domains among the β subunits were exchanged indicated that this region of the integrin subunit appears to contribute to the differences in integrin utilizations among strains. In contrast, the G-H loops of the different viruses do not appear to be involved in this phenomenon. Thus, the ability of the virus to utilize multiple integrins in vitro may be a reflection of the use of multiple receptors during the course of infection within the susceptible host.

Foot-and-mouth disease virus (FMDV) is one of the most feared viral pathogens of livestock. Outbreaks can result in high morbidity and loss of production in infected animals, but the most devastating economic consequence to affected countries results from mass livestock culling and international trade restrictions imposed on animals and animal products. FMDV is the type species of the *Aphthovirus* genus of the *Picornaviridae* family and exists as many subtypes and variants within seven different serotypes.

Limited trypsin digestion of FMDV results in the generation of noninfectious virions that are unable to adsorb to susceptible cells (5) due to the cleavage of VP1 at the Arg (R) residue of a highly conserved Arg-Gly-Asp (RGD) motif (62) located within a flexible external loop between the βG and βH strands (G-H loop) (1, 36, 41). In addition, peptides containing the RGD sequence inhibited the adsorption of the virus to tissue culture cells (6, 21), and genetically engineered virions containing either mutations or deletions of the RGD sequence were unable to bind to cells or cause disease in susceptible animals (37, 45, 46). These observations have led to the conclusion that the virus utilizes cell surface integrin molecules as receptors via this RGD site. Subsequently, it has been demonstrated that at least three integrins, $\alpha_V\beta_1$, $\alpha_V\beta_3$, and $\alpha_V\beta_6$, can serve as receptors for FMDV in vitro (10, 32, 33, 52, 53).

Integrins are heterodimeric type I membrane glycoproteins composed of two subunits (α and β) that interact noncovalently at the cell surface (30). They mediate cell-cell interactions and the binding of cells to the extracellular matrix, and in doing so, they play a crucial role in cell division, differentiation, migration, and survival (23). The eighteen α and eight β mammalian integrin subunits can assemble into 24 different heterodimers (29). While the consensus binding motif of some integrins is unknown, at least eight integrins recognize and bind to ligands via an RGD sequence (63). Interestingly, despite recognizing this tripeptide sequence, these integrins bind different extracellular matrix ligands (40, 69). While the reasons for this specificity are obscure, there is evidence that ligand sequences either flanking the RGD motif or located in other regions of the ligand are responsible for binding specificity (12, 54). One subgroup within the integrin family is the α_V integrins, comprising five heterodimers ($\alpha_V\beta_1$, $\alpha_V\beta_3$, $\alpha_V\beta_5$, $\alpha_V\beta_6$, and $\alpha_V\beta_8$), all of which recognize the RGD motif on their natural ligands (30, 63).

While the aforementioned observations strongly suggest that RGD-dependent integrins probably direct FMDV to target tissues during a natural infection in susceptible hosts, the fact that the virus can utilize multiple α_V integrins in vitro leaves open the question of how these different receptors may function in determining viral pathogenesis. To begin to answer this question, we molecularly cloned the bovine β_1, β_5, and β_6 integrin subunits and, using previously cloned bovine α_V and β_3 subunits (52), compared α_V integrin receptor utilizations among several different representatives of FMDV serotypes A and O. Surprisingly, we found that viruses of these two serotypes utilized these integrins with different efficiencies. We also

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TABLE 1. Primers used in cloning and sequencing of bovine integrin β subunits

Primer no.	Sequence (5′ → 3′)	Subunit(s)	Orientation	Source(s)[a]	Use[b]
34	CTGGATTGGACTGATCAGTTC	β_1	Forward	Human, cat	Sequencing
35	GCAAATGCCAAATCATGTGGAG	β_1	Forward	Human	Sequencing
36	CTCATACTTCGGATTAAC	β_1	Reverse	Bovine	Sequencing
37	GTGTCCCATTTGGCATTC	β_1	Reverse	Bovine	Sequencing
9	GGTCTCAACATATGCACTAGTG	β_5	Forward	Human	Sequencing
11	CCGGTCGTGGATGGTGAC	β_5	Reverse	Human, bovine	Sequencing
38	GTACAAGGTGGCTGTGCC	β_6	Forward	Human	Sequencing
80	GGTACCTCTAGATTATCAACTGGGGGCGGTCCCACAC	β_5	Reverse		Cloning
40	TCCCGTTTGCCACTTGGC	β_6	Reverse	Human, murine	Sequencing
84	GGTCTAGACTCGAGCCGCGGGAGAAGATG	β_1	Forward		Cloning
71	TCCACAGACACACTCTCC	β_1	Reverse		Cloning
70	TTGATCCCTAAGTCAGCG	β_1	Forward		Cloning
97	GGAATTCTTAAGGTACCTTATCATTTTCCCTCATACTTCGGATTAAC	β_1	Reverse		Cloning
91	GGTCTAGACTCGAGGGCGCCCCACCATGCCG	β_5	Forward		Cloning
31	GCTGCAAATGTGGCCATC	β_5	Reverse		Cloning
30	CCCTGGTCAGAGGAAGTG	β_5	Forward		Cloning
92	GGAATTCTTAAGCTTATCAGTCCACTGTGCCATTG	β_5	Reverse		Cloning
161	TCTAGACTCGAGCTGAAACGGATGGGGATT	β_6	Forward		Cloning
162	CTTAAGGGTACCTTACTATCCATCCGTGGAAAG	β_6	Reverse		Cloning
124	CAGATTACCCAAGTCAGTCCACAGGAGGTCACGGTG	β_3,β_5	Forward		LBD exchange
125	ATCCAGCGCTATATGGGTCACGTCGTCCGTGGTAAA	β_3,β_5	Reverse		LBD exchange
126	GATGTCATCCAGCTGACACCCCAGAGGATTGCCCTC	β_5,β_3	Forward		LBD exchange
127	GTCCAGTGCGATGTGGGGCTTGGCATCAGTGGTAAA	β_5,β_3	Reverse		LBD exchange
128	CAGATTACCCAAGTCAGTCCACAGCAGTTGGTTCTG	β_3,β_1	Forward		LBD exchange
129	ATCCAGCGCTATATGGGTCACAGCATCTGTGGAAAA	β_3,β_1	Reverse		LBD exchange
130	GATATCACTCAAATCCAGCCCCAGAGGATTGCCCTC	β_1,β_3	Forward		LBD exchange
131	ATCTCCAGCGAAGTGAAACTTGGCATCAGTGGTAAA	β_1,β_3	Reverse		LBD exchange
173	CAGATTACCCAAGTCAGTCCTCAAAGCTTGGCTCTT	β_3,β_6	Forward		LBD exchange
176	ATCCAGCGCTATATGGGTGTCCGCGTCACTCACAAA	β_3,β_6	Reverse		LBD exchange
174	GACATCGTTCAGATTTCACCCCAGAGGATTGCCCTC	β_6,β_3	Forward		LBD exchange
175	GTCCATTCCAAAATGAGACTTGGCATCAGTGGTAAA	β_6,β_3	Reverse		LBD exchange

[a] The GenBank accession numbers of the sequences used to derive the primers are given in the text. Where there is no source listed, the primer was generated using sequence determined during the cloning of the subunit.
[b] See Materials and Methods.

examined the roles of the β subunit ligand-binding domains (LBDs) and the G-H loops of the different viruses in α_V integrin utilization.

MATERIALS AND METHODS

Sequencing of bovine integrin β_1, β_5, and β_6 subunits. Sequencing of the bovine integrin subunits was performed prior to molecular cloning in order to ascertain the exact sequences of the 5′ and 3′ ends. The primers used for cloning and sequencing of the integrin subunits are listed in Table 1. Total RNA was extracted from primary bovine tongue keratinocytes (55) (for the β_1 and β_5 subunits) and from secondary calf thyroid cells (for the β_6 subunit) by using Trizol (Invitrogen) as described by the manufacturer. cDNA was amplified from total RNA, which was primed with oligo(dT), by using SuperScriptII (Invitrogen). All PCR amplifications were performed with *Pfu* polymerase (Stratagene). For the amplification of the β_1 subunit, the forward primers 34 and 35 (Table 1) were selected from alignments of the GenBank human and feline β_1 integrin sequences X07979 and U27351, respectively, and the reverse primers 36 and 37 (Table 1) were selected from the GenBank partial bovine sequence U10815. A 2.2-kbp β_1 fragment was amplified by PCR with primers 34 and 36 followed by a nested PCR with primers 35 and 37. For the amplification of the β_5 subunit, forward primer 9 and reverse primer 11 (Table 1) were selected from consensus sequences in the alignment of human and murine sequences (GenBank accession numbers J05633 and AF022110, respectively) and used to amplify a 2.1-kbp specific β_5 fragment by PCR. For the amplification of the β_6 subunit, forward primer 38 and reverse primer 40 (Table 1), selected from alignments of the human and murine β_6 sequences (GenBank accession numbers NM000888 and AF115376, respectively), were used to amplify a 2.2-kbp fragment by PCR. The PCR products were inserted into the Zero Blunt TOPO vector (Invitrogen), as described by the manufacturer, and sequenced on an ABI 3700 DNA analyzer (Applied Biosystems) by using an ABI Prism Big Dye terminator cycle-sequencing ready reaction kit (Perkin-Elmer). The central sequences of these bovine integrin genes were used to design primers for the amplification and sequencing

of the 5′ and 3′ ends by using a 5′/3′ RACE kit (Roche Molecular Biochemicals) as described by the manufacturer. Sequence analysis was done using the Lasergene analysis software package (DNASTAR Inc.).

Assembly of complete cDNA clones of bovine integrin β_1, β_5, and β_6 subunits. Two PCR amplicons, together comprising the complete integrin β_1 subunit and containing some overlapping sequences, were amplified from cDNA prepared from bovine tongue keratinocyte RNA (see above) by using the primer pairs 84-71 and 70-97 (Table 1). Both amplicons were cloned independently into pCR-Blunt II-TOPO (Invitrogen) and assembled into a complete cDNA by using the restriction sites *Cla*I and *Xma*I. The assembled cDNA was transferred into the mammalian expression vector pcDNA3.1/zeo(−) (Invitrogen) to create pBovβ1 by using the restriction sites *Xba*I and *Hind*III included in the amplification primers. The β_5 subunit was amplified from cDNA prepared from bovine tongue keratinocytes (see above) by using the primer pairs 91-31 and 30-92 (Table 1), assembled into pCR-Blunt II TOPO by using the *Bgl*II and *Xma*I sites, and transferred into pcDNA3.1/zeo(−) by using the restriction sites *Xba*I and *Hind*III to create pBovβ5. The bovine β_6 subunit was amplified as a single amplicon by using cDNA prepared from secondary bovine thyroid cells (see above) and primers 161 and 162 (Table 1). This amplicon was ligated into pcDNA3.1/zeo(−) by using the *Kpn*I and *Xho*I sites to create pBovβ6. The complete integrin subunit cDNAs were resequenced and analyzed by coupled in vitro transcription-translation using the rabbit reticulocyte TNT Quick Coupled system (Promega) as described previously (52). Plasmids pBovα_VZEO and pBovβ_3ZEO have been described previously (52).

Viruses and cells. FMDV type A_{12} strain 119ab (A_{12}) was derived from the infectious cDNA clone pRMC$_{35}$ (61). The cDNA was assembled from a virus with an unknown high-passage history in both bovine kidney and BHK-21 cells and which, following recovery from transfected BHK-21 cells, has been passaged numerous times in cattle. The virus exhibits mild virulence in this cell type. An antigenic variant of type A_{12} (A_{12}-SSP), harboring the VP1 sequence present in a bovine tongue tissue-derived virus, was assembled into a cDNA (vRM-SSP) as described previously (59). Following recovery from transfected BHK-21 cells, the virus was passaged twice in CHO cells expressing an engineered receptor con-

TABLE 2. Viruses used in this study[a]

Virus	Derivation	Passage in tissue culture	Virulence in cattle
A_{12}	Infectious clone $pRMC_{35}$ (61)	Multiple passages in BHK-21 cells	Mild
A_{12}-SSP	Infectious clone pRM-SSP containing VP1 sequence from bovine tongue tissue virus (59)	Two passages in CHO cells expressing scab/ICAM1 receptor (60)	Not tested
A_{24}Cru	Foot lesion of experimentally infected bovine	None	Virulent
O_1Camp	Infectious clone pCRM8 (64)	Less than five passages in BHK-21 cells	Virulent
O/Taw/2/99	Esophagopharyngeal fluid from bovine (27)	Three passages in BHK-21 cells, one passage in bovine thyroid cells, and two passages in BHK-21 cells	Subclinical infection (27, 28)
A/O	Infectious clone pRM-A/O; type A_{12} virus with G-H loop from type O_1BFS virus (58)	Multiple passages in BHK-21 cells	Not tested

[a] Numbers in parentheses indicate references.

sisting of a single-chain anti-FMDV antibody fused to intercellular adhesion molecule 1 (scAb/ICAM1) (60). The virulence of this virus in cattle has not been tested. FMDV type A_{24}Cruziero (A_{24}Cru) was recovered from a foot lesion of a steer experimentally inoculated with virus intradermally into the tongue. The virus was used directly from the vesicular fluid and not passaged in tissue culture. The cattle-virulent variant of type O_1Campos (O_1Camp) was derived from the infectious cDNA clone pCRM8, which contains capsid sequences isolated from a vaccine seed stock and has been described previously (64). FMDV O/Taw/2/99 was isolated from the esophagopharyngeal fluid of a bovine with a subclinical FMDV infection (27). The virus was passaged three times in BHK-21 cells in Taiwan and then sent to the Institute for Animal Health, Pirbright, United Kingdom, where it was passaged once in primary bovine thyroid cells. It was then sent to the Plum Island Animal Disease Center, where it was passaged twice in BHK-21 cells. This virus did not cause clinical disease when it was experimentally inoculated into the species of cattle from which it was originally isolated (27, 28), nor did it cause clinical disease when inoculated into cattle at the Plum Island Animal Disease Center, but all animals seroconverted (P. W. Mason, personal communication). The genetically engineered type A_{12} virus chimera, where the G-H loop has been replaced with the homologous loop sequences from type O_1BFS (A/O) virus, has been described previously (58). A summary of the properties of these viruses is presented in Table 2.

BHK-21 cells were maintained in minimum essential medium containing 10% calf serum and 10% tryptose phosphate broth. COS-1 cells were maintained in Dulbecco's minimum essential medium (Life Technologies) containing 10% fetal calf serum, an additional 2 mM concentration of L-glutamine, and 1 mM sodium pyruvate.

Exchanging putative LBDs among β subunits. The putative LBDs of the β_1, β_5, and β_6 integrin subunits were substituted into the β_3 subunit, and the β_3 LBD was substituted into the β_1, β_5, and β_6 subunits. The exchanges were accomplished by a three-step recombinant PCR method that resulted in clean swapping of the LBDs into the paralogous integrin backbones cloned in the pcDNA3.1/zeo(−) background. The LBD to be exchanged was amplified with the specific primers (listed in Table 1) containing 5′ extensions, comprising approximately one-half the length of the primer, corresponding to the integrin subunit contributing the backbone sequences. The primer 5′ extensions allowed the PCR-mediated insertion of the LBD amplicons to backbone integrin fragments upstream and downstream of the LBD. The recombinant fragment containing the heterologous LBD was inserted into plasmids expressing the backbone integrin by digestion with appropriate restriction enzymes. The chimeric full-length cDNAs were designated $p\beta_3[\beta_1]$, $p\beta_3[\beta_5]$, $p\beta_5[\beta_3]$, $p\beta_1[\beta_3]$, $p\beta_3[\beta_6]$, and $p\beta_6[\beta_3]$, where the first β designation corresponds to the donor of the integrin subunit backbone and the subunit in brackets corresponds to the LBD donor. All chimeric β subunit cDNAs were totally resequenced to confirm the presence of the exchanged regions and to rule out the introduction of other mutations. Plasmids were also analyzed by in vitro transcription-translation as described above.

Transient expression of integrin subunits in COS-1 cells and viral replication assays. Expression of integrin subunits in COS-1 cells and analysis of viral replication were performed as described previously (52). Briefly, cells plated on six-well tissue culture plates were transfected with 2 μg each of pBovaα_VZEO and the appropriate β subunit cDNA plasmid by using the transfection reagent FuGENE6 (Roche Molecular Biochemicals). Transfected cells were incubated overnight, followed by trypsinization and replating onto 24-well plates. After a further overnight incubation, transfected cultures were infected with FMDV type A_{12}, A_{24}Cru, O_1Camp, or O/Taw/99 at a multiplicity of infection of 10 PFU/cell or A_{12}-SSP at a multiplicity of infection of 1 PFU/cell. Infected cells were labeled with [^{35}S]methionine between 4 and 18 h postinfection, and viral replication was

determined on infected cell lysates by radioimmunoprecipitation (RIP) of equal amounts of trichloroacetic acid-precipitable counts per minute of lysate by using monoclonal antibody (MAb) 6EE2 directed against FMDV type A_{12} (8) or 10GA4 directed against type O_1 (67) as described previously (52). Immunoprecipitated proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 12.5% polyacrylamide gel. Transfected cells in one of the wells were not infected but were analyzed for integrin expression by immunohistochemistry as described previously (52) by using MAbs LM609, 6S6, and CSβ6 directed to the human integrins $\alpha_V\beta_3$, β_1, and β_6, respectively, or a polyclonal rabbit serum directed against the bovine β_5 subunit.

Nucleotide sequence accession numbers. The nucleotide and amino acid sequences for the bovine β_1, β_5, and β_6 integrin subunit cDNAs have been deposited in GenBank and assigned the accession numbers AF468058, AF468059, and AF468060, respectively.

RESULTS

Cloning and sequence analysis of bovine integrin β subunits. At least three different α_V integrins, $\alpha_V\beta_1$, $\alpha_V\beta_3$, and $\alpha_V\beta_6$, have been reported to function as FMDV receptors in vitro (10, 32, 33, 53), and Neff et al. have previously shown that the virus utilized bovine $\alpha_V\beta_3$ more efficiently than its human homolog (52). In preliminary experiments using K562 cells stably transfected with human $\alpha_V\beta_5$, we were unable to demonstrate receptor utilization of that integrin by FMDV type A_{12} (unpublished observation). In light of the aforementioned observations, we cloned and analyzed the cDNAs encoding bovine β_1, β_5, and β_6 subunits. The β_1 and β_5 subunit-coding sequences were amplified from cDNA prepared from primary bovine tongue keratinocytes, while sequences coding for the β_6 subunit were amplified from cDNA prepared from secondary bovine thyroid cell cultures (see Materials and Methods).

The complete coding sequence for the bovine β_1 subunit comprised 2,397 nucleotides coding for a 798-amino-acid-residue protein, consisting of a 20-residue signal peptide, a 708-residue ectodomain, a 29-residue transmembrane domain, and a 41-residue cytoplasmic domain. The coding sequence for the β_5 subunit comprised 2,403 nucleotides coding for an 800-amino-acid-residue protein, consisting of a 23-residue signal peptide, a 697-residue ectodomain, a 29-residue transmembrane domain, and a 51-residue cytoplasmic domain. A comparison with the human β_5 sequence revealed that the bovine homolog contained one additional codon located within the ectodomain. The coding sequence for the β_6 subunit comprised 2,367 nucleotides coding for a 788-amino-acid-residue protein consisting of a 26-residue putative signal peptide, a 681-residue ectodomain, a 29-residue transmembrane domain, and a 52-residue cytoplasmic domain.

TABLE 3. Nucleotide and amino acid similarities between bovine
and human integrin β subunits

Subunit	% Nucleotide similarity/ % amino acid similarity in[a]:				
	Mature subunit[b]	Signal peptide	Ecto-domain	Transmembrane domain	Cytoplasmic domain
β_1	89.2/94.1	87.3/90.0	88.8/93.5	94.3/100	92.9/100
β_5	88.1/92.0	85.3/79.2	88.0/91.5	84.9/89.7	89.9/100
β_6	88.4/93.4	91.4/62.1	88.3/93.0	87.4/96.6	89.3/98.0

[a] The first number of each pair represents the sequence similarity of nucleotides aligned using the Martinez/Needleman-Wunsh alignment method and determined using a gap penalty of 1.10 and a gap length penalty of 0.33. The second number represents the sequence similarity of amino acids aligned using the Lipman-Pearson alignment method and determined using a gap penalty of 4 and a gap length penalty of 12. The putative boundaries between the domains of the bovine subunits were defined from alignments to the human homologs and the reported borders of the domains in the human sequences.
[b] Sequence comparison of the complete ectodomain, transmembrane domain, and cytoplasmic domain.

The nucleotide and predicted amino acid sequence similarities within the different subunit functional regions among the human and bovine β subunits are shown in Table 3. Consistent with the previously analyzed bovine and human α_V and β_3 homologs (52), the greatest degree of amino acid sequence divergence of the mature β_1 and β_6 subunits occurred within the ectodomain. The β_5 subunit exhibited the highest amino acid sequence divergence within the ectodomain of the three subunits and, surprisingly, had a higher degree of amino acid sequence divergence within the transmembrane domain (Table 3). The amino acid sequences of the cytoplasmic domain were totally conserved for the β_1 and β_5 subunits, but there was a single codon change in the β_6 subunit. The cytoplasmic domain of the human β_6 subunit has been shown to be required for the integrin to function as a receptor for FMDV (49), which is in contrast to the bovine β_3 subunit, where deletion of almost the entire cytoplasmic domain does not affect the viral receptor function of the integrin (51). Characteristic of all integrin β subunits is the high content of cysteine residues and four tandem cysteine-rich epidermal growth factor (EGF)-like domains known as the cysteine-rich repeats (24). Alignments of the human and bovine predicted amino acid sequences of the β_1, β_5, and β_6 subunits showed that all cysteines were conserved, with the exception of a C → Y change in the bovine β_1 ectodomain at residue 671, just downstream of the last EGF-like domain. Neff et al. have previously found a C → R substitution in the bovine β_3 subunit within the second EGF-like domain (52).

Replication of type A_{12} and O_1Camp viruses in COS-1 cells expressing bovine integrins. COS-1 cells were cotransfected with pBovα_VZEO and pBovβ_1, pBovβ_3ZEO, pBovβ_5, or pBovβ_6 as described in Materials and Methods. Cells were monitored for integrin expression by immunohistochemistry (52). More than 80% of the cells in the cultures transfected with $\alpha_V\beta_3$, $\alpha_V\beta_5$, or $\alpha_V\beta_6$ cDNA stained positively for the appropriate integrin (data not shown). COS-1 cells, however, express the β_1 subunit naturally, possibly as the $\alpha_5\beta_1$ heterodimer. Since the available anti-human α_V MAbs did not cross-react with the bovine homolog, we were unable to effectively monitor $\alpha_V\beta_1$ expression in these cultures. The integrin expression in the transfected cells was always tested in parallel cell culture preparations in the subsequent FMDV

infection experiments, and only the results of experiments where at least 80% of the cells expressed the proper integrins are reported.

Transfected and nontransfected COS-1 cultures were infected with either FMDV type A_{12} or type O_1Camp to evaluate integrin usage preference between these two serotypes. Both viruses have been demonstrated to utilize both bovine and human $\alpha_V\beta_3$ as receptors, although they utilized the bovine integrin more efficiently (52, 53). In addition, neither virus utilizes heparan sulfate as a receptor, as has been demonstrated for some tissue culture-adapted FMDV strains (31, 53, 64). Infected cells were labeled with [^{35}S]methionine between 4 to 18 h after infection, and viral replication was analyzed by RIP as described in Materials and Methods. In this and subsequent experiments, only cells originating from the same transfected culture were infected with the different viruses. In addition, within each strain, equal numbers of trichloroacetic acid-precipitable counts per minute were immunoprecipitated, allowing direct comparison of viral replication between cultures expressing different integrins. The results of a typical transfection-infection assay are shown in Fig. 1a. Under these conditions, virus-specific proteins could not be detected in nontransfected cells infected with type A_{12} virus. In contrast, low levels of virus-specific proteins were present in nontransfected cells infected with type O_1Camp virus. This finding, which has been observed previously (52), may be due to contaminating virus which can utilize heparan sulfate as a receptor. In cells transfected with cDNAs encoding the bovine integrins and infected with either type A_{12} or O_1Camp virus, virus-specific proteins were detected in all the integrin-transfected cell cultures. However, the level of viral protein synthesis in type A_{12} virus-infected cells expressing either $\alpha_V\beta_3$ or $\alpha_V\beta_6$ was much greater than that in cells transfected with either $\alpha_V\beta_1$ or $\alpha_V\beta_5$. In contrast, type O_1Camp virus appeared to utilize the $\alpha_V\beta_6$ integrin more efficiently than any of the other integrins and utilized $\alpha_V\beta_1$ more efficiently than either $\alpha_V\beta_3$ or $\alpha_V\beta_5$.

To quantitate the level of viral replication in the transfected cells, we digitized the autoradiogram and quantitated the intensities of the VP1 bands relative to the intensity determined for nontransfected cells. $\alpha_V\beta_3$ and $\alpha_V\beta_6$ were equally efficient in mediating infection by type A_{12} virus, while $\alpha_V\beta_1$ and $\alpha_V\beta_5$ were poor receptors for this virus (Fig. 1b). In contrast, replication of type O_1Camp virus was mediated by $\alpha_V\beta_6$ and $\alpha_V\beta_1$ to a greater degree than by either $\alpha_V\beta_3$ or $\alpha_V\beta_5$ (Fig. 1c).

To determine whether the transfected cells became susceptible to FMDV as a specific consequence of integrin expression, we pretreated transfected cells with function-blocking anti-integrin MAbs against human $\alpha_V\beta_3$, $\alpha_V\beta_6$, or $\alpha_V\beta_1$, followed by infection with either type A_{12} or O_1Camp virus in the presence of the antibodies. Viral replication in either $\alpha_V\beta_1$- or $\alpha_V\beta_3$-transfected cells was inhibited in the presence of the appropriate antibody (data not shown). Treatment with the available anti-$\alpha_V\beta_6$ blocking MAb resulted in a low degree of inhibition of viral replication (data not shown). While this antibody has been demonstrated to inhibit the replication of a type O_1 virus in cells transfected with human $\alpha_V\beta_6$ (33), we found by using flow cytometry that it reacts poorly with the bovine integrin (data not shown). The available function-blocking antibodies against human $\alpha_V\beta_5$ did not cross-react
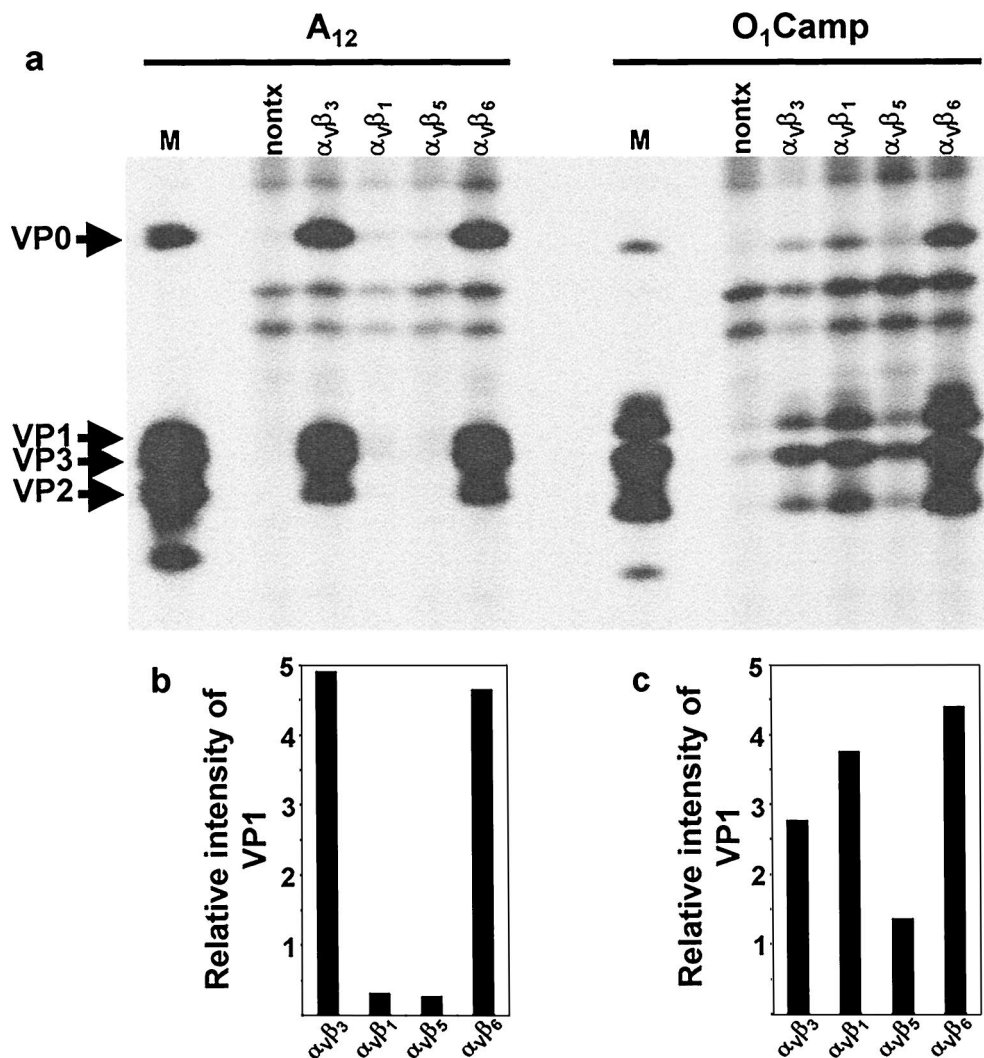
FIG. 1. Viral replication in type A_{12} or O_1Camp virus-infected COS-1 cells expressing bovine integrins. COS-1 cells were cotransfected with cDNA plasmids encoding the bovine integrin α_V subunit and the β_1, β_3, β_5, or β_6 subunit. (a) Transfected cells were infected with FMDV type A_{12} or O_1Camp and labeled with $[^{35}S]$methionine, and viral protein synthesis was analyzed by RIP and SDS-PAGE as described in Materials and Methods. Lanes: nontx, immunoprecipitated proteins from nontransfected infected cell lysates; M, the locations of the viral structural proteins from lysates prepared from FMDV-infected BHK-21 cells are indicated. (b and c) The autoradiogram shown in panel a was digitized in a MultiImage Light Cabinet, and the intensities for VP1 bands in cells infected with type A_{12} (b) or type O_1Camp (c) virus were quantitated with the spot density utility of AlphaEase software, version 4.0. The bars represent the intensities of the VP1 band in transfected cells relative to that of the same region in nontransfected cells.

with the bovine integrin, so we were unable to perform this study with cells transfected with this integrin.

Role of the β subunit LBD in mediating virus infection. The LBD of the intact integrin consists of discrete regions of both the α and β subunits (20, 74). To analyze what role, if any, the β subunit LBD plays in distinguishing different virus strains, we exchanged homologous regions corresponding to the putative LBDs of the individual subunits, which resulted in a series of β subunit chimeras, as described in Materials and Methods. These chimeras were cotransfected with the bovine α_V subunit into COS-1 cells, followed by infection with either type A_{12} or O_1Camp virus. Viral replication mediated by the chimeras was analyzed as described in Materials and Methods and compared to replication mediated by the wild-type integrins, and a representative experiment is shown in Fig. 2.

In transfected cells infected with type A_{12} virus, replacement of the LBD of the β_3 subunit with the LBD of either the β_1 or β_5 subunit reduced the efficiency of the $\alpha_V\beta_3$ integrin as a receptor. Conversely, replacing the β_1 LBD with the β_3 LBD did not increase the efficiency of the $\alpha_V\beta_1$ integrin as a receptor for this virus; however, replacing the β_5 LBD with the β_3 LBD resulted in a small increase in the efficiency of the $\alpha_V\beta_5$ integrin. Exchanging LBDs of the β_1 and β_3 subunits appeared to lower the efficiencies of the β_1 and β_3 subunits in mediating type O_1Campos virus infection, similar to what was observed with type A_{12} virus. Likewise, exchanging the LBDs of the β_3 and β_5 subunits resulted in a decrease in β_3 receptor efficiency and a small increase in β_5 receptor efficiency. Interestingly, while exchange of the LBDs of the β_3 and β_6 integrin subunits lowered the efficiencies of both of these integrins as viral re-
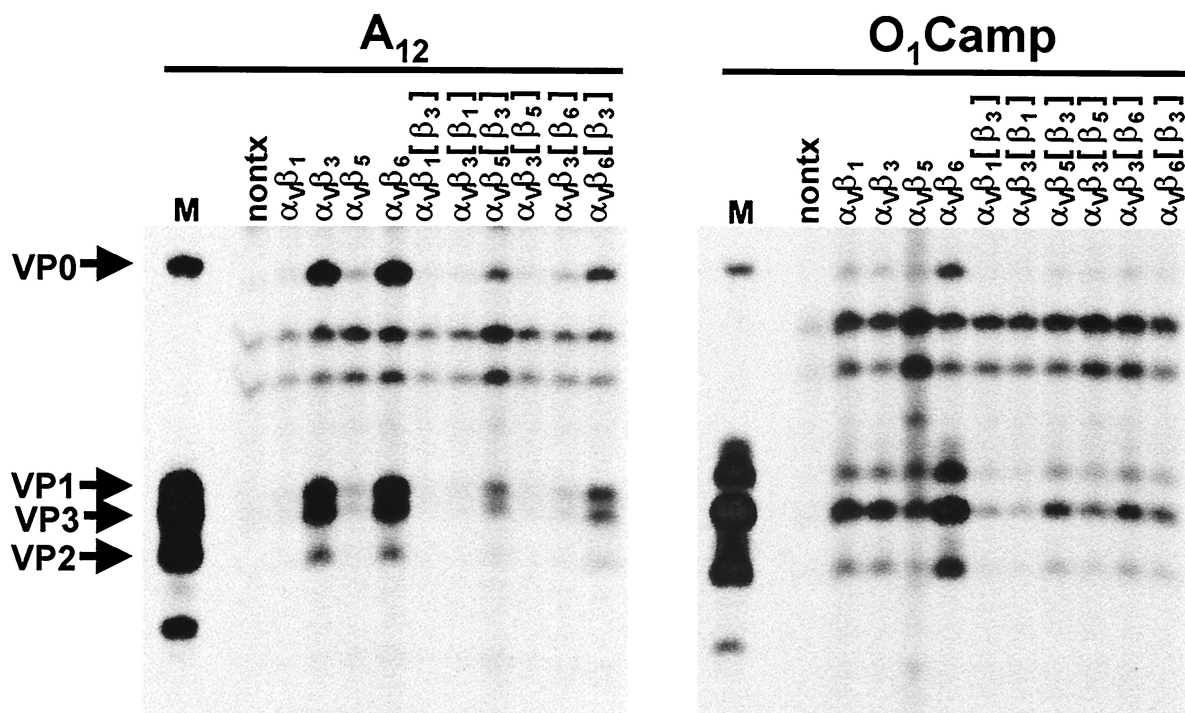
FIG. 2. Viral replication in COS-1 cells expressing β subunit LBD chimeras. Cells were cotransfected with cDNA plasmids encoding the bovine α_V subunit and either wild-type β subunits or β subunit LBD chimeras as indicated in the figure and infected with either type A_{12} or O_1Camp virus. Viral replication was analyzed by RIP and SDS-PAGE as described in Materials and Methods. Lanes: nontx, immunoprecipitated proteins from nontransfected infected cell lysates; M, the locations of the viral structural proteins from lysates prepared from FMDV-infected BHK-21 cells are indicated.

ceptors for both types A_{12} and O_1Camp, type A_{12} virus utilized the $\beta_6[\beta_3]$ subunit and type O_1Camp virus utilized the $\beta_3[\beta_6]$ subunit slightly more efficiently than the subunits with the reciprocal exchanges.

Analysis of integrin utilization by other type A and O viruses. In order to ascertain whether the preference for different integrins was unique to types A_{12} and O_1Camp or was common to the individual serotypes, we analyzed viral replication in cells transfected with each of the cloned integrin cDNAs of two additional type A viruses and one additional type O virus. These viruses and their passage histories are described in Materials and Methods and summarized in Table 2. The results of this experiment are shown in the autoradiogram in Fig. 3a, and the quantitation is shown in Fig. 3b and c. All of the type A viruses efficiently utilized the $\alpha_V\beta_3$ integrin as a receptor. Types A_{12} and A_{24}Cru also utilized the $\alpha_V\beta_6$ integrin efficiently as a receptor, while type A_{12}-SSP does so to a lesser extent. Surprisingly, A_{24}Cru also utilized the $\alpha_V\beta_1$ integrin as a receptor with relatively high efficiency. In contrast, the two type O viruses utilized only the $\alpha_V\beta_6$ integrin with high efficiency and utilized $\alpha_V\beta_1$ slightly better than $\alpha_V\beta_3$.

Influence of the G-H loop on α_V integrin utilization. The above-mentioned results indicate that the viral serotype, not the tissue culture history or the relative bovine virulence of the individual isolates, appeared to determine the efficiency of integrin utilization. While there could be many factors which determine the integrin specificity of the individual serotypes, we focused on the G-H loop. A comparison of the sequences of

the loops for all of the viruses utilized in this study (Fig. 4) showed that the sequences are more conserved within the serotype than between the serotypes. All of the loops have a conserved Tyr near the N terminus of the loop and a conserved Leu-Ala at the RGD + 4 position. The two type O viruses have a Leu at the RGD + 1 position, and the DLXXL sequence has been suggested to be an $\alpha_V\beta_6$ recognition sequence (35). The most striking difference between the serotypes is the presence of a Cys at the base of the loop in the type O viruses which forms a disulfide bond with a Cys residue in VP2 (41, 56). The type A viruses do not have this disulfide bond, allowing the G-H loop to assume a conformation, relative to the rest of the capsid, that is different from what is seen in the type O viruses (16). Reduction of the disulfide bond in the type O_1 G-H loop results in the rearrangement of the loop (41) into a conformation similar to that seen in the type A viruses (16). To examine the role of the loop in integrin differentiation, we transfected COS-1 cells with cDNA encoding either $\alpha_V\beta_3$ or $\alpha_V\beta_6$, followed by infection with type A_{12} or O_1Camp virus that was treated with 10 mM dithiothreitol (DTT) or a type A_{12} virus chimera where the G-H loop was replaced with sequences representing the G-H loop of type O_1BFS (58). Treatment of either type A_{12} or O_1Camp virus with DTT did not reduce the infectivity of the virus, as determined by plaque assay in BHK-21 cells (data not shown), confirming previously reported results (41). Our results, shown in Fig. 5, indicate that neither treatment with DTT nor replacement of the type A_{12} G-H loop
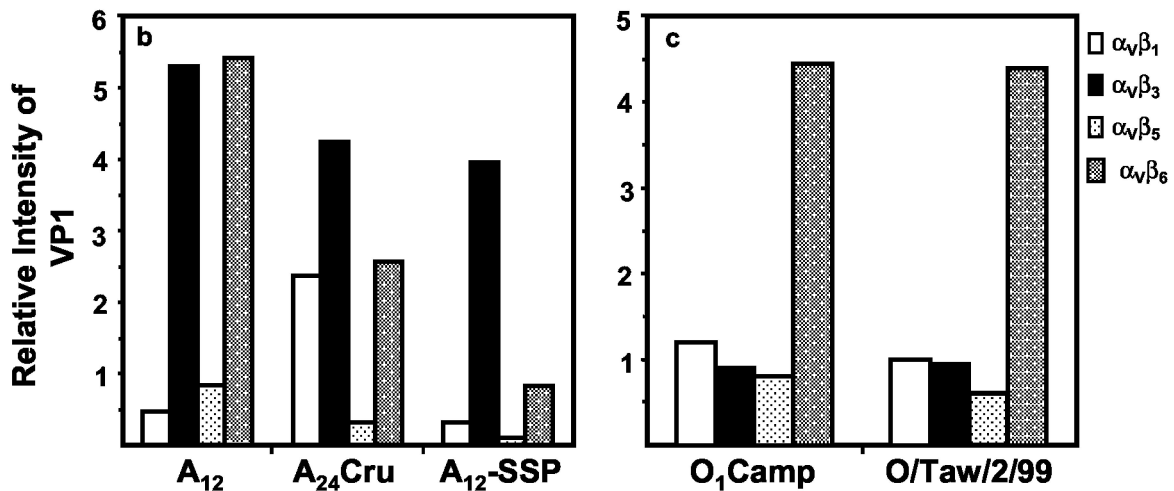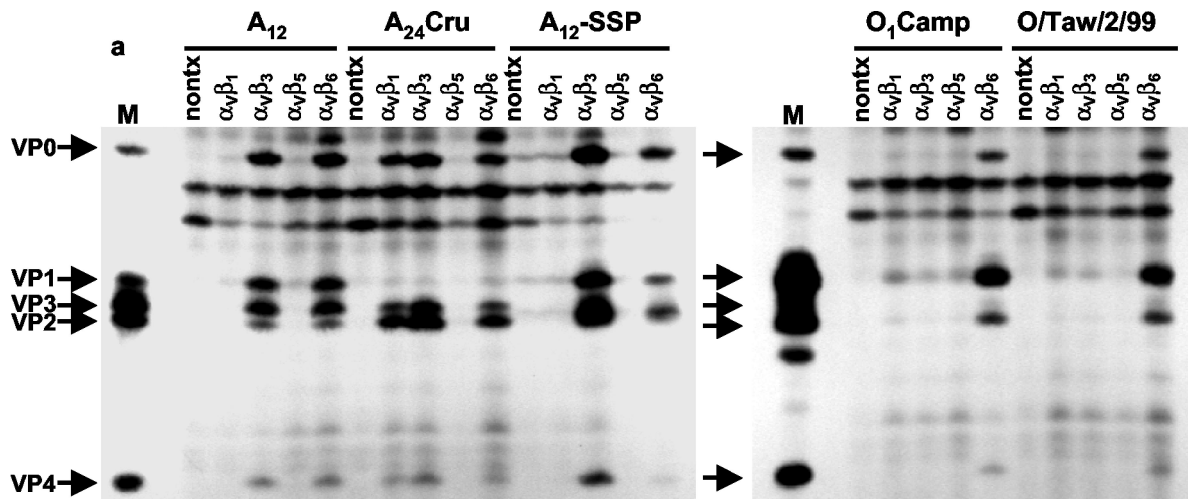
FIG. 3. Viral replication in FMDV-infected COS-1 cells expressing bovine integrins. COS-1 cells were cotransfected with cDNA plasmids encoding the bovine integrin α_V subunit and the β_1, β_3, β_5, or β_6 subunit. (a) Transfected cells were infected with different FMDV types as noted in the figure and labeled with [^{35}S]methionine, and viral protein synthesis was analyzed by RIP and SDS-PAGE as described in Materials and Methods. Lanes: nontx, immunoprecipitated proteins from nontransfected infected cell lysates; M, the locations of the viral structural proteins from lysates prepared from FMDV-infected BHK-21 cells are indicated. (b and c) The autoradiogram shown in panel a was digitized in a MultiImage Light Cabinet, and the intensities for VP1 bands in cells infected with type A (b) or type O (c) viruses were quantitated with the spot density utility of AlphaEase software, version 4.0. The bars represent the intensities of the VP1 band in transfected cells relative to that of the same region in nontransfected cells.

with a type O_1 loop had any dramatic effects on the differentiation of the two receptors by either type A_{12} or O_1Camp virus.

DISCUSSION

In addition to integrins, FMDV has been shown to utilize various cell surface molecules as receptors in vitro. These include Fc receptors (7, 43, 45), heparan sulfate (4, 31, 42, 53), and a single-chain antibody fused to intercellular adhesion molecule 1 (60). Since the original identification of the $\alpha_V\beta_3$ integrin as a receptor for FMDV (10, 52, 53), two additional integrins, $\alpha_V\beta_6$ (33) and $\alpha_V\beta_1$ (32), have been shown to function as FMDV receptors in cell culture. The present report expands on these earlier studies by using molecularly cloned

A_{12}	tnkYsas-gsgv*RGD*fgsLAprva
A_{24}Cru	tskYavg-gsgr*RGD*mgsLAarvv
A_{12}-SSP	tnkYsas-gsgv*RGD*sgsLAprva
O_1Camp	ecrYsrnavpnv*RGD*lqvLAqkva
O/Taw/2/99	nckYgespvtnv*RGD*lqvLAqkaa

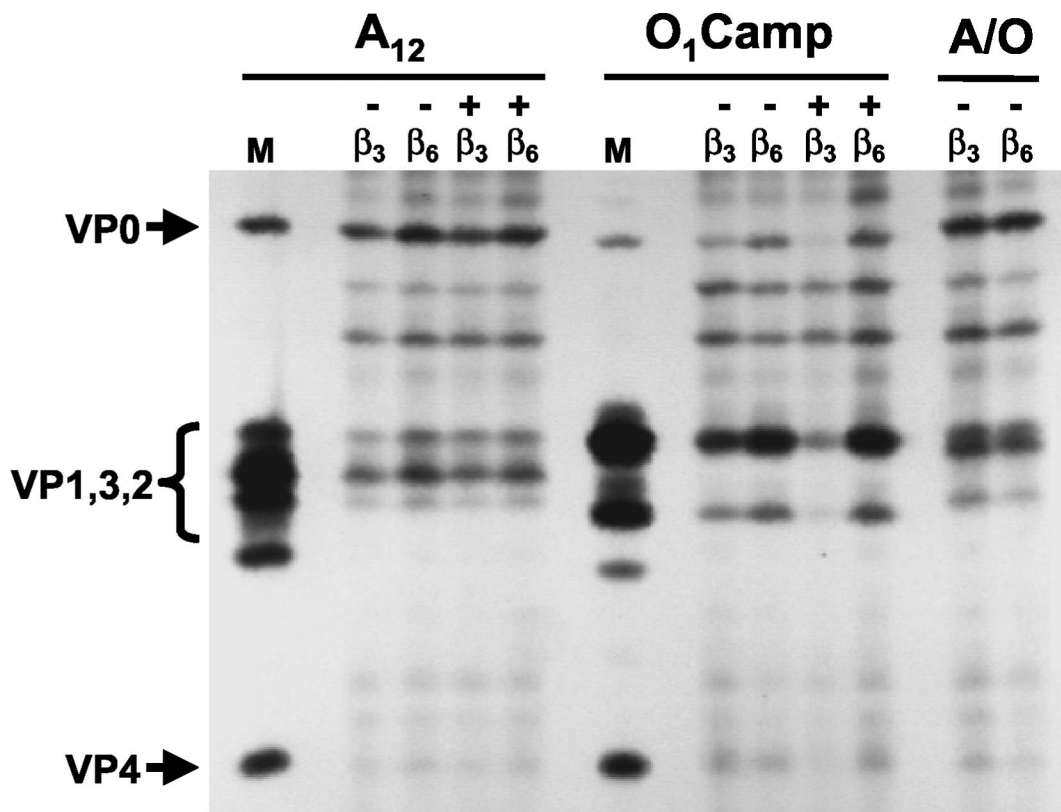FIG. 4. Sequences of the G-H loops of type A and O viruses.

FIG. 5. Replication of DTT-reduced FMDV or an FMDV G-H loop chimera in COS-1 cells expressing $\alpha_V\beta_3$ or $\alpha_V\beta_6$. COS-1 cells were cotransfected with cDNA plasmids encoding the bovine integrin α_V subunit and either the β_3 or β_6 subunit. FMDV type A_{12} or O_1Camp was incubated with 10 mM DTT for 30 min at room temperature. Transfected cells were immediately infected with either DTT-treated (+) or untreated (−) FMDV or the type A/O G-H loop chimera as noted in the figure and labeled with [^{35}S]methionine, and viral protein synthesis was analyzed by RIP and SDS-PAGE as described in Materials and Methods. Lanes M, the locations of the viral structural proteins from lysates prepared from FMDV-infected BHK-21 cells are indicated.

bovine integrin subunits and demonstrates differences in α_V integrin receptor utilizations by different FMDV strains.

We employed a transient integrin expression assay system in COS-1 cells (51, 52) to compare the efficiencies of each of the α_V integrins in mediating infection by strains representing two different FMDV serotypes. In each experiment, the transfected cells used for infection originated from the same transfected culture and all transfections and infections were done at the same time. Thus, we minimized the degree of variation that may result from differences in the expressions of the different integrins in the transient expression assay system. Our results demonstrated that the efficiencies by which these four bovine integrins are able to mediate infection differed between the two virus serotypes. While the type O viruses utilized $\alpha_V\beta_6$ with the highest efficiency, followed in descending order by $\alpha_V\beta_1$, $\alpha_V\beta_3$, and $\alpha_V\beta_5$, all of the type A viruses utilized $\alpha_V\beta_3$ with high efficiency and utilized $\alpha_V\beta_6$ with equal or lesser efficiency. Only one type A virus (A_{24}Cru) utilized $\alpha_V\beta_1$ with moderate efficiency, and all utilized $\alpha_V\beta_5$ very poorly. These results, obtained by using integrins from a species susceptible to FMDV, confirmed the finding of Jackson et al. (32, 33) that type O_1 FMDV utilizes $\alpha_V\beta_6$ and $\alpha_V\beta_1$ as receptors in vitro.

While interactions between the α and β subunits contribute to the LBD of integrins, specific regions of both subunits have

been shown to interact directly with ligands (20). Since the same α subunit was present in all of the integrins in this study, we concentrated on differences in the β subunits as possible explanations for differences in receptor specificities between the two viruses. To further define the reasons for the observed specificities among the β subunits, we exchanged regions of the subunits' putative LBDs. The regions we chose to exchange have been identified functionally as LBDs by various biochemical criteria, and exchanging these regions among β subunits resulted in changes in the ligand specificities of the integrins (11, 18, 40, 69, 70). The recently solved crystal structure of the $\alpha_V\beta_3$ integrin, which has led to the structural definition of the integrin's LBD, showed that the region exchanged in this study interacts with the α subunit within the ligand-binding cleft and contains the RGD binding site, but the entire LBD of the β subunit appears to encompass a larger region of the ectodomain than that exchanged in this study (74, 75). In all cases, however, removing even this portion of the LBD from its natural context and replacing it with an LBD of another β subunit lowered the efficiency of the integrin as a receptor for FMDV. The only exception appeared to be in the β_5 subunit, where replacing the β_5 LBD with the β_3 LBD resulted in a slight increase in the efficiency of the $\alpha_V\beta_5$ receptor. When the LBDs for the β_3 and β_6 subunits were exchanged, we observed

that the type A_{12} virus utilized the β_6 subunit containing the β_3 LBD with higher efficiency than the β_3 subunit with the β_6 LBD. Conversely, the O_1Camp virus utilized the β_3 subunit with the β_6 LBD with slightly higher efficiency than the β_6 subunit with the β_3 LBD. These results suggest that the β subunit LBD plays a role in the recognition of the different viral serotypes; however, with some of the chimeric constructs, we were unable to directly measure integrin expression since reactivity with the anti-integrin MAbs appeared to be either reduced or abolished. Thus, some of the differences in viral replication could be related to differences in the expressions of the chimeric integrins on the cell surface. Neff et al. have previously shown that the increased efficiency of the bovine β_3 subunit compared with that of the human homolog as a receptor for FMDV appeared to map to the EGF-like cysteine-rich repeat region downstream of the LBD (52), raising the possibility that differences in receptor specificities for FMDV among the integrins may be related to either the LBD or the LBD and other regions of the subunit ectodomain.

The five viruses we analyzed in this study have different degrees of virulence in the bovine (Table 2). The A_{24}Cru and O_1Camp viruses are virulent to bovines and cause a rapid and severe clinical disease upon exposure, while the A_{12} and O/Taw/2/99 viruses cause either a mild or subclinical disease. The A_{12}-SSP virus has not been tested directly in animals. This suggests that differences in receptor recognition do not appear to be related to the virus' ability to cause disease but rather to the serotype of the virus. Eight integrins recognize RGD as a binding motif sequence on their natural ligands (63). Since each of these integrins displays unique ligand specificities, other regions of the ligands are likely to influence recognition by the receptor. It is interesting that the G-H loops of the type A viruses used in this study contain a conserved leucine at the RGD + 4 position but lack a leucine at the RGD + 1 position (Fig. 4). The DLXXL motif has been shown to be involved in recognition of the $\alpha_V\beta_6$ integrin (35). These viruses, however, utilize the $\alpha_V\beta_6$ integrin quite efficiently. Thus, the differences in receptor specificities displayed by the type A and O viruses in vitro may be the result of the amino acid sequence divergence within the G-H loop surrounding the RGD sequence (Fig. 4), differences in loop structure, or interactions with other capsid regions outside of the G-H loop. To examine the first possibility, we made use of an engineered chimeric type A virus in which the G-H loop of a type O virus (O_1BFS) was substituted for the type A loop (58). The sequence of the substituted loop is identical to that of the G-H loop from type O_1Camp virus, with the exception of a Val → Leu change at the RGD − 1 position. This chimeric virus appears to utilize $\alpha_V\beta_3$ and $\alpha_V\beta_6$ to the same extent as does the wild-type A_{12} virus (Fig. 5). This result indicates that the amino acid residues immediately surrounding the RGD sequence in the G-H loop do not appear to greatly influence the receptor utilization by the virus, although we have not generated and tested a type O virus with a type A loop.

To analyze the influence that the conformation of the G-H loop has on receptor utilization, we infected cells expressing either $\alpha_V\beta_3$ or $\alpha_V\beta_6$ with type O_1Camp virus that was pretreated with DTT. In type O viruses, the base of the G-H loop is linked, via a disulfide bond, between a Cys at residue 134 and a Cys at residue 130 of VP2 (56). Reduction of this disulfide

bond by DTT results in a rearrangement of the G-H loop within the viral particle, allowing for the determination of the loop structure (41). For the type A viruses, only the structure of the type A_{22} virus has been determined, and within the crystal, the G-H loop was disordered (16). Type A viruses, however, do not contain the Cys residue at the base of the G-H loop in VP1, and the structure of the residues at the base of the loop was very similar to that seen in the structure of DTT-treated, but not native, type O_1 virus (16). In addition, DTT treatment of type O_1 virus resulted in a shift of the G-H loop of VP3 (41) into a conformation almost identical to that seen in the type A_{22} particle (16). These results suggested that there are differences in the dispositions of the G-H loop between type A and O viruses, and these differences are eliminated by reduction of the disulfide bond in type O viruses. It has also been shown that the disulfide bond is reduced in newly released virus, suggesting that the reduced loop conformation may be biologically relevant (41). We therefore reasoned that by placing the G-H loop of type O_1 virus into a more type A-like conformation, the virus would resemble the type A viruses in its receptor utilization. The results in Fig. 5 indicate, however, that treatment of the type O_1 virus with DTT did not affect receptor usage. In fact, the reduced virus utilized $\alpha_V\beta_3$ to a lesser extent than did the native virus. Treatment of type A_{12} virus with DTT did not affect receptor utilization. In this experiment, we did not further modify the type O virus to prevent reformation of the disulfide bond; however, DTT was present during the entire adsorption period. Experiments examining the reoxidation of the disulfide bond in vitro have estimated that the half time for reformation of the bond at room temperature is 4 days (41). Thus, it appears that, since neither the primary sequence nor the structural conformation of the loop play a role in the relative utilization of the $\alpha_V\beta_3$ and $\alpha_V\beta_6$ integrins by types A and O, other regions of the viral capsid must be involved in this interaction.

Little information pertaining to the viral receptors important in the pathogenesis of FMDV in susceptible species is available. If the virus uses only one of the RGD-dependent integrins in vivo, then a correlation should exist between the sites where the virus replicates and causes the lesions and the tissue distribution of that particular integrin. The other possibility is that more than one integrin is used during different stages of the disease. In this scenario, the initial stages of infection of an aerosol-infected animal could involve the utilization of receptors in the upper respiratory tract, whereas later replication cycles and amplification in epithelial sites in the mouth and feet could involve the utilization of alternate receptors. While it appears that the disease process in susceptible animals is mediated by virus-integrin interactions (46, 53, 64), a type C virus containing an RGGD sequence has been isolated from a bovine which was not protected from virus challenge following immunization with an experimental peptide vaccine (68). In addition, a tissue culture-adapted type C virus with a genetically engineered RGG sequence, which was unable to bind to heparin, replicated in BHK-21 cells which express both heparan sulfate and FMDV integrin receptors and in heparan sulfate-negative CHO cell mutants which do not express FMDV integrin receptors (2, 3). The ability of these viruses to cause disease in susceptible animals, however, has not been demonstrated. More recently, a tissue culture-

adapted derivative of a Cathay topotype O_1 virus isolated in China has also been shown to replicate in tissue culture in both a heparan sulfate- and integrin-independent manner and cause mild disease in swine (76). However, it should be stressed that, with the exception of the virus isolated from a bovine (68), the other viruses were obtained through either tissue culture adaptation or genetic engineering. Thus, the possible role of nonintegrin receptors in FMDV pathogenicity needs to be more closely examined.

While there are many reports on the distribution of some of the α_V integrins in human tissues, little information on the tissue expression of the integrins in FMDV-susceptible animals is available. In human tissues, the $\alpha_V\beta_3$ integrin is found on vascular endothelium and smooth muscle (15, 19, 39) but is not found on bronchiolar epithelium (47). This integrin has been shown to be expressed in an estrous cycle-dependent manner in bovine endometrial epithelium (34) and is expressed weakly in bovine and porcine airway epithelium (65). In contrast, the $\alpha_V\beta_6$ integrin is restricted to epithelial cells (14) but is rarely found in normal tissues in humans (13, 25, 72, 73). High levels of $\alpha_V\beta_6$ have also been found in the macula densa of the kidneys and the endometrial epithelium of secretory phase uterus (14). In general, the expression of $\alpha_V\beta_6$ is highly regulated and is found during development, healing processes, and neoplasia (13). There is very little information available on the expression or function of the $\alpha_V\beta_1$ integrin in specific tissues or organs, although this integrin is expressed on malignant cells (22, 48), in smooth muscle (17), and in the central nervous system (50). It has also been demonstrated to be a receptor for human parechovirus 1 (57, 71) and a coreceptor for human adenovirus (38). The β_1 subunit forms dimers with at least 10 different α subunits, making the β_1 integrins the largest subgroup within the integrin family (30). However, only three of the β_1 integrins, $\alpha_V\beta_1$, $\alpha_5\beta_1$, and $\alpha_8\beta_1$, utilize the RGD sequence as a binding recognition motif (63).

Obtainment of information on integrin expression in susceptible animals would allow some correlation to be made between integrin use by the virus in cell culture and sites of virus replication in vivo. The tissues we utilized for integrin cloning gave us some indication of integrin expression in cattle. cDNAs for the β_1 and β_5 subunits were easily amplified from RNA extracted from primary bovine tongue keratinocytes. We have also been able to amplify both α_V and β_3 cDNAs from these keratinocytes by PCR (unpublished data). In contrast, we were unable to amplify sequences coding for the β_6 subunit from cDNAs prepared from a number of bovine tissues collected at necropsy, including tongue epithelium, tongue keratinocytes, lung, and kidney. This was surprising given that some of these organs are targets for the virus during natural infection. We were able, however, to amplify cDNA encoding this integrin subunit from RNA prepared from secondary cultures of bovine thyroid. These cells are considered to be highly sensitive to FMDV infection and are often used for primary isolation of virus from field samples (26, 66). The role of the thyroid, if any, in FMDV pathogenesis in vivo is not known.

While this report has concentrated on the role of the viral receptor in pathogenesis, other viral and cellular factors may also affect both host range and virulence (44). In particular, it has recently been demonstrated that attenuation of virulence in bovines and reduced ability to replicate in bovine cells are associated with deletions in the nonstructural protein 3A (9, 55) At this time, it is difficult to reconcile why this virus would utilize at least three different receptors to cause disease. While infection with different FMDV strains may result in different degrees of disease severity, in most cases there are no apparent differences in clinical symptoms within a species. There are, however, differences in the clinical courses of disease among the different species which are susceptible to FMDV. Thus, it is possible that these may be related to differences in either expression patterns or degrees of expression of the known integrin receptors for FMDV among different species. Analysis of the distribution of the integrin receptors in susceptible species may be necessary to explain viral pathogenesis within different species.

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