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# Specific Interactions between Human Integrin $\alpha_v\beta_3$ and Chimeric Hepatitis B Virus Core Particles Bearing the Receptor-Binding Epitope of Foot-and-Mouth Disease Virus

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Purified integrin $\alpha_v\beta_3$ was used in solid-phase binding studies with chimeric hepatitis B cores which carry the RGD-containing loop of VP1 protein of the foot-and-mouth disease virus (FMDV). High levels of specific binding between the integrin and the particles were detected by enzyme-linked immunosorbent assays. The binding was $Mn^{2+}$ cation dependent and could be competed with fibronectin, vitronectin, and the peptide GRGDSPK. Particles in which the RGD motif had been mutated to RGE failed to bind, indicating that the chimeric cores bound specifically to the ligand binding site of integrin $\alpha_v\beta_3$. Electron micrographs showed several individual $\alpha_v\beta_3$ molecules bound to the surface of each chimeric particle. Collectively, these data constitute firm evidence that the RGD-containing loop of FMDV is critical for binding to $\alpha_v\beta_3$ and provide support for identification of $\alpha_v\beta_3$ as a potential cellular receptor for FMDV. 

## INTRODUCTION

Cell–cell and cell–extracellular matrix adhesive interactions are essential phenomena in the biology of all organisms and a variety of molecules are responsible for these functions. Integrins are possibly the largest and most complex of the adhesion molecules identified so far (Giancotti and Mainiero, 1994; Hynes, 1992; Ruoslahti and Pierschbacher, 1987). Adhesive interactions mediated by integrins are essential in many biological processes such as cell differentiation, immune recognition, immune response, and blood coagulation. Integrins are heterodimeric molecules that are formed by the noncovalent association of $\alpha$ and $\beta$ subunits; the molecular weight of these heterodimers is greater than 200 kDa. The integrin $\alpha_v\beta_3$ is involved in angiogenesis and in control of cell migration, and it plays a significant role in atherosclerosis, tumor metastasis, and osteoporosis (Hynes, 1992; Giancotti and Mainiero, 1994; Albelda *et al.,* 1990; Friedlander *et al.,* 1995). Various ligands which bind to it include adhesive proteins bearing an RGD[2] (Arg-Gly-Asp) tripeptide, such as vitronectin, fibronectin, osteopontin, fibrinogen, collagen, laminin, neural cell adhesion molecule L1, thrombospondin, and von Willebrand factor (Hynes, 1992; Montgomery *et al.,* 1996). In addition, $\alpha_v\beta_3$ binds to CD31 (also called platelet endothelial cell adhesion molecule or PECAM), a member of the immunoglobulin superfamily which lacks the RGD sequence in any of its six extracellular domains (Buckley *et al.,* 1996; Piali *et al.,* 1995).

The promiscuity of ligand acceptance by integrins has been exploited by a number of viruses which use them as receptors at some stage in the infection process and this has long been suspected to be the case for foot-and-mouth disease virus (FMDV). The first indication that FMDV uses integrins came from comparative sequence data which showed that the motif RGD is highly conserved at a position in the capsid protein, VP1, for which there was biochemical evidence of involvement in receptor binding (Geysen *et al.,* 1985). Furthermore, cell attachment by the virus could be competed by synthetic peptides bearing the RGD motif (Fox *et al.,* 1989), a feature indicative of an integrin-ligand interaction. Additionally, structural studies had shown that the RGD triplet was located within a highly mobile loop (the $\beta$G–$\beta$H loop comprising residues 134–159 of the $\beta$ barrel core of VP1

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[2] Abbreviations used: RGD, Arg-Gly-Asp; RGE, Arg-Gly-Glu; $\beta$-OG, $\beta$-octyl glucoside; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; EM, electron microscope; ICRF, Imperial Cancer Research Fund; Fn, fibronectin; FMDV, foot-and-mouth disease virus; HRP, horseradish peroxidase; ICAM, intercellular adhesion molecule; PBS, phosphate-buffered saline; OPD, *o*-phenylenediaminedihydrochloride; TBS, Tris-buffered saline; Vn, vitronectin.

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protein) at the surface of FMDV (Fox *et al.,* 1989; Acharya *et al.,* 1989); the $\beta$G–$\beta$H loop had been shown to be in an open configuration typical of integrin ligands (Logan *et al.,* 1993). The importance of this motif for receptor binding was confirmed by the demonstration that mutation of the sequence to RGE via an infectious clone of FMDV A12 abolished cell attachment and infectivity (Mason *et al.,* 1994).

Competition binding experiments using anti-integrin monoclonal antibodies suggested that the molecule used as a receptor by FMDV A12 is $\alpha_v\beta_3$ (Berinstein *et al.,* 1995). Indeed, recently it has been shown that several serotypes (including O1) of FMDV bind to the integrin $\alpha_v\beta_3$ *in vitro* (Jackson *et al.,* 1997) via the RGD-containing loop (the same loop has been used in this work to generate the chimeric particles). However, it is possible that different isolates of FMDV may have similar, but not identical, receptor binding specificities since in radiolabeling competition assays heterologous virus does not usually compete as well as homologous virus for receptor binding (Sekiguchi *et al.,* 1982). The receptor-binding specificity may be modulated by regions of the virus surface other than the RGD-containing VP1 $\beta$G–$\beta$H loop since removal of only the C-terminal portion of VP1 has also been shown to compromise cell attachment (Fox *et al.,* 1989; Rieder *et al.,* 1994b). Recently, heparan sulfate has been identified as a primary receptor for the virus (Jackson *et al.,* 1996) and it remains unclear to what extent receptor specificity is achieved by each of the two receptors.

To examine further the role of the VP1 $\beta$G–$\beta$H loop of FMDV serotype O1 in determining receptor binding and specificity, a DNA sequence coding for this peptide was inserted into the e1 loop region of the hepatitis B virus core protein (Chambers *et al.,* 1996). Such fusion proteins readily assemble into virus-like particles (FM.e1-HBc) when expressed in bacteria and display an array of the introduced sequence on their surface, thus resembling the natural presentation of the feature on FMDV (Chambers *et al.,* 1996). These particles can elicit high levels of FMDV-neutralizing antibodies in guinea pigs and compete with virus for binding to cells in culture (Chambers *et al.,* 1996).

In this paper we describe features of the binding of FM.e1-HBc particles to purified preparations of integrin $\alpha_v\beta_3$ attached to solid surfaces. We also show electron micrographs of the integrin molecules bound to the surface of the recombinant FM.e1-HBc particles.

## MATERIALS AND METHODS

### Peptides and antibodies

An integrin binding peptide GRGDSPK (Pytela *et al.,* 1987) was synthesized at the Imperial Cancer Research Fund (ICRF) facility (London) and used for competition experiments. For integrin purification and solid-phase binding experiments a mouse monoclonal antibody 23C6 that recognizes the functional $\alpha_v\beta_3$ heterodimer was used (supplied by ICRF).

### Purification of the integrin $\boldsymbol{\alpha_v\beta_3}$

Integrin $\alpha_v\beta_3$ was purified from human placental extracts using antibody-affinity chromatography with Sepharose-conjugated 23C6, similar to published protocols (Smith and Cheresh, 1988; Pfaff *et al.,* 1994). Briefly, a human placenta was homogenized in a commercial blender in the presence of 50 mM Tris–Cl, pH 7.5, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 150 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 1 $\mu$g/ml each of leupeptin, pepstatin, and antipain (lysis buffer). The mix was allowed to incubate at 4° for 1 h and was then spun in a benchtop centrifuge to clear the tissue debris and unlysed material. The lysate was then spun in an ultracentrifuge for 1 h at 30,000 rpm and the supernatant loaded on a protein A–Sepharose column to remove the considerable amounts of immunoglobulin present in placental material. Flowthrough was collected and loaded on the 23C6 antibody column (approximately 30 mg of 23C6 antibody was coupled to activated Sepharose). The column was washed with 5 column volumes of lysis buffer, with and subsequently without Triton X-100. Acidic buffer (20 mM NaAc, pH 3.1, and 30 mM $\beta$-OG) was used to elute the integrin, and 1-ml fractions were collected and neutralized simultaneously using 2 M Tris–Cl, pH 8.0. These fractions were run on a 9% SDS–PAGE gel and visualized by Coomassie blue staining. Purified $\alpha_v\beta_3$ was judged $>$90% pure by SDS–PAGE gels and was stored at 5 mg/ml in 50 mM Tris–Cl, pH 7.5, 1 mM $MnCl_2$, 0.1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 150 mM NaCl, and 30 mM $\beta$-OG at 4°. A sample of the purified $\alpha_v\beta_3$ along with molecular weight markers is shown in Fig. 1.

### FM.e1-HBc purification

Expression and purification of chimeric FM.e1-HBc (RGD containing), mutant FM.e1-HBc (RGE containing), and HBc (wild-type cores) was performed as detailed previously (Chambers *et al.,* 1996). Briefly, the native and chimeric HBc proteins were overexpressed in a bacterial expression system and the particles purified by sucrose density gradient centrifugation methods (Chambers *et al.,* 1996). Amino acid sequence of the FMDV loop inserted in chimeric cores is 135-RYSRNAVPNLRGDLQVLAQKV-ARTLP-160 (Chambers *et al.,* 1996).

### Solid-phase binding assay

Solid-phase binding assays were performed as follows: Purified FM.e1-HBc particles and other control pro-

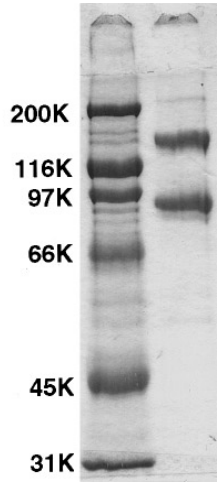


FIG. 1. SDS–PAGE of purified placental integrin $\alpha_v\beta_3$. 20-$\mu$l samples of eluted fractions from the antibody column 23C6 were run on a 9% SDS–polyacrylamide gel under nonreducing conditions. Lane 1, broad-range molecular weight markers in daltons (Bio-Rad): 200,000 (top), 116,000, 97,400, 66,000, 45,000, and 31,000; lane 2, the integrin receptor $\alpha_v\beta_3$ showing the two subunits, $\alpha_v$ at ~150,000 and $\beta_3$ at ~90,000 daltons.

teins were diluted in 50 mM carbonate–bicarbonate, pH 9.6, buffer (Sigma) to a concentration of 5–10 $\mu$g/ml and 100 $\mu$l was added to a 96-well Nunc-Maxisorp ELISA plate (Gibco, Life Technologies). These microtiter plates were incubated overnight at 4°, washed three times with 0.25% PBS (wash buffer), and blocked with 200 $\mu$l of 2% BSA–PBS for 2 h at room temperature. Wells were again washed three times with the wash buffer and 100 $\mu$l of the purified integrin $\alpha_v\beta_3$ (~0.1–4 $\mu$g/well) was added to each well. In competition/inhibition experiments, GRGDSPK peptide or other known ligands of integrin $\alpha_v\beta_3$ were added simultaneously. To assess the effects of divalent cations the integrin and antibody proteins were diluted in TBS with 1 mM $MnCl_2$, 0.1 mM $MgCl_2$, and 0.1 mM $CaCl_2$ except for data shown in Fig. 3, for which the integrin was diluted in TBS only. After incubation for 2 h at room temperature the wells were washed three times. A 1:5000 dilution of the 23C6 antibody (stock was 1 mg/ml) was then added in 100-$\mu$l aliquots to each well and incubated for 30 min at room temperature followed by a 1:1000 dilution of a goat anti-mouse antibody–HRP conjugate (stock was 1 mg/ml) for the same time period. Wells were finally washed four times, color was developed using the OPD substrate (Sigma), and OD was measured at 450 nm.

### Electron microscopy

Samples were prepared for negative staining as follows: 1 $\mu$g of FM.e1-HBc particles was incubated with approximately 10 $\mu$g of purified $\alpha_v\beta_3$ in 50 mM Tris–Cl, pH 7.5, 1 mM $MnCl_2$, 0.1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 150 mM NaCl, and 30 mM $\beta$-OG at room temperature for 1 h. The complex was then spotted onto a carbon-coated grid, negatively stained with 2% uranyl acetate solution, and visualized in a JEOL 100 CX (at 100 kV) electron microscope.

## RESULTS

### Bacterially produced chimeric FM.e1-HBc particles bearing the $\beta$G–$\beta$H loop of VP1 protein bind to purified human integrin $\alpha_v\beta_3$

Human integrin $\alpha_v\beta_3$ was affinity purified to homogeneity as judged by SDS–PAGE (Fig. 1) by using the heterodimer-specific monoclonal antibody 23C6 (Buckley *et al.,* 1996). With the use of appropriate monoclonal antibodies it was possible to demonstrate the presence of the $\alpha_v$, $\beta_3$, and heterodimer $\alpha_v\beta_3$ chains, but not of $\beta_1$, $\beta_5$, or $\alpha_{1-6}$ (data not shown). The binding of purified integrin $\alpha_v\beta_3$ to the recombinant FM.e1-HBc particles or to other control proteins was assessed by ELISA in which the potential ligands were bound to a solid surface. The FM.e1-HBc particles bound the $\alpha_v\beta_3$ with similar dose response to the natural ligands, fibronectin and vitronectin, as shown in Fig. 2A. No significant binding was seen with either of two control proteins, namely, Fc-ICAM1 and Fc-Muc18 (these are fusion proteins comprising the immunoglobulin Fc region fused to the ICAM1 or Muc18 extracellular domains). In competition assays both fibronectin and vitronectin reduced the $\alpha_v\beta_3$/FM.e1-HBc binding to background levels (Fig. 2B). This suggests that the recombinant FM.e1-HBc particles bind to $\alpha_v\beta_3$ as the natural ligands do.

### Cation requirements for the binding of $\alpha_v\beta_3$ to FM.e1-HBc particles

Divalent cations are essential for integrin–ligand interactions and play an important role in regulating the consequences of such interactions (Pytela *et al.,* 1987). The effects of cations such as $Ca^{2+}$, $Mg^{2+}$, and $Mn^{2+}$ range from activation of ligand binding to inhibition of ligand binding and, possibly, modulation of ligand specificity (Hynes, 1992; Gailit and Ruoslahti, 1988; Hu *et al.,* 1995; Kirchhofer *et al.,* 1990; Smith *et al.,* 1994; Suehiro *et al.,* 1996). To investigate further the binding of FM.e1-HBc particles to $\alpha_v\beta_3$ in the context of divalent cations, ELISAs were performed in the absence of divalent cations or in the presence of 1 mM $Ca^{2+}$, $Mg^{2+}$, or $Mn^{2+}$. As shown in Fig. 3A, FM.e1-HBc particles bind to $\alpha_v\beta_3$ only in the presence of 1 mM $Mn^{2+}$, which is consistent with reports on the activation of integrins by $Mn^{2+}$. As expected from the divalent cation dependence of integrin–ligand interactions, the FM.e1-HBc/$\alpha_v\beta_3$ binding was abrogated in the presence of 5 mM EDTA (data not shown).

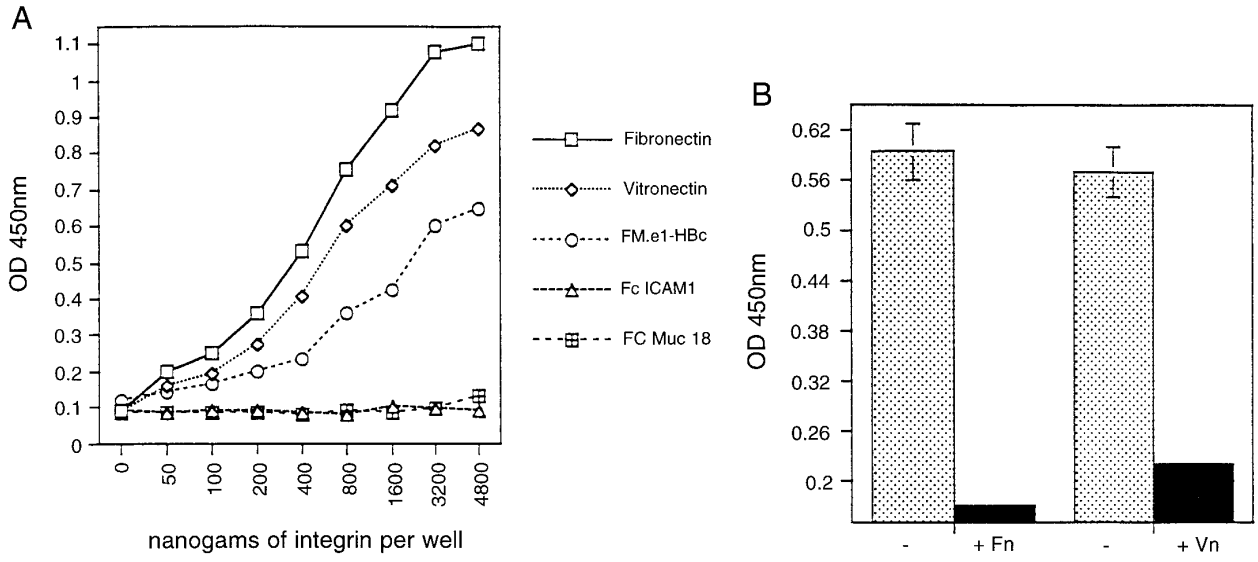


FIG. 2. Dose–response and competition experiments. Integrin binding to FM.e1HBc cores was detected as described under Materials and Methods. (A) Fibronectin, vitronectin, FM.e1-HBc particles, and Fc-ICAM1 and Fc-Muc18 (control proteins) were coated on plastic and increasing amounts of the integrin $\alpha_v\beta_3$ added to each well in the concentration range shown. (B) FM.e1-HBc particles were used to coat microtiter plates and then incubated with integrin $\alpha_v\beta_3$ with (indicated with a + sign) or without (indicated with a − sign) 1 $\mu$g/well soluble fibronectin (Sigma) or 0.5 $\mu$g/well vitronectin (Sigma). Both experiments were done in triplicate and values shown were averaged (where error bars are not shown the standard deviation was less than 10% of the mean).

### FM.e1-HBc/$\alpha_v\beta_3$ interaction inhibited by an RGD-containing peptide

Within the integrin family the $\beta_3$ integrins show an apparent lack of specificity toward their ligands outside of the RGD motif (Suehiro *et al.,* 1996). Specifically, the integrin $\alpha_v\beta_3$ has been shown to bind to up to eight ligands and recently more have been identified (Hynes, 1992; Montgomery *et al.,* 1996; Buckley *et al.,* 1996; Piali *et al.,* 1995). To verify whether the FM.e1-HBc/$\alpha_v\beta_3$ interaction was indeed based on the RGD loop of FMDV, and to confirm the specificity of this interaction, inhibition studies with a seven-residue RGD peptide (GRGDSPK) were done. As shown in Fig. 3B the GRGDSPK peptide inhibited binding between FM.e1-HBc and $\alpha_v\beta_3$ in a dose-dependent manner and complete inhibition was achieved with 50 $\mu$M peptide. Additionally, FM.e1-HBc particles in which the RGD sequence was mutated to an RGE did not bind to the integrin $\alpha_v\beta_3$ in solid-phase assays (Fig. 3C). These data confirm that FM.e1-HBc/$\alpha_v\beta_3$ binding is indeed via the RGD-containing loop of FMDV.

### Visualization of FM.e1-HBc particles complexed with integrin $\alpha_v\beta_3$

To address the structural nature of FM.e1-HBc/$\alpha_v\beta_3$ interaction, potential complexes of FM.e1-HBc/$\alpha_v\beta_3$ were spotted on carbon-coated grids, negatively stained, and visualized in the EM. The diameter of FM.e1-HBc particles was estimated from such electron micrographs to be ~300 Å (see Fig. 4A), and the diameter of the globular head of $\alpha_v\beta_3$ has previously been determined to be ~80 Å (A.S., unpublished observations). A distinct decoration of the surface of FM.e1-HBc particles with ~80-Å diameter $\alpha_v\beta_3$ molecules could be seen in the electron micrographs (Fig. 4B). Variable numbers of integrin molecules were seen on different core particles and no unattached 80-Å structures were seen, suggesting that the concentration of the $\alpha_v\beta_3$ was subsaturating. The complexes were necessarily formed in 30 mM OG since at lower concentrations of the detergent the hydrophobic tails of integrin $\alpha_v\beta_3$ molecules associated to form dimers, trimers, and rosettes (A.S., unpublished observations), as has been seen with the platelet receptor integrin (Nermut *et al,* 1988). In light of (a) the biochemical data presented here, (b) the fact that purified components (FM.e1-HBc particles and $\alpha_v\beta_3$) were incubated and coated on the carbon-coated grids, and (c) the consistency in molecular size of individual components and the FM.e1-HBc/$\alpha_v\beta_3$ complexes, it can be concluded that the molecules that decorate the surface of the FM.e1-HBc particles are specifically bound integrin $\alpha_v\beta_3$.

## DISCUSSION

Insertion of the RGD-containing loop from FMDV into HBc protein confers on the resulting assembled particles (FM.e1-HBc) the ability to bind efficiently to cells and to $\alpha_5\beta_1$ integrin *in vitro* (Chambers *et al.,* 1996). Here we show that the chimeric particles can also bind to $\alpha_v\beta_3$,

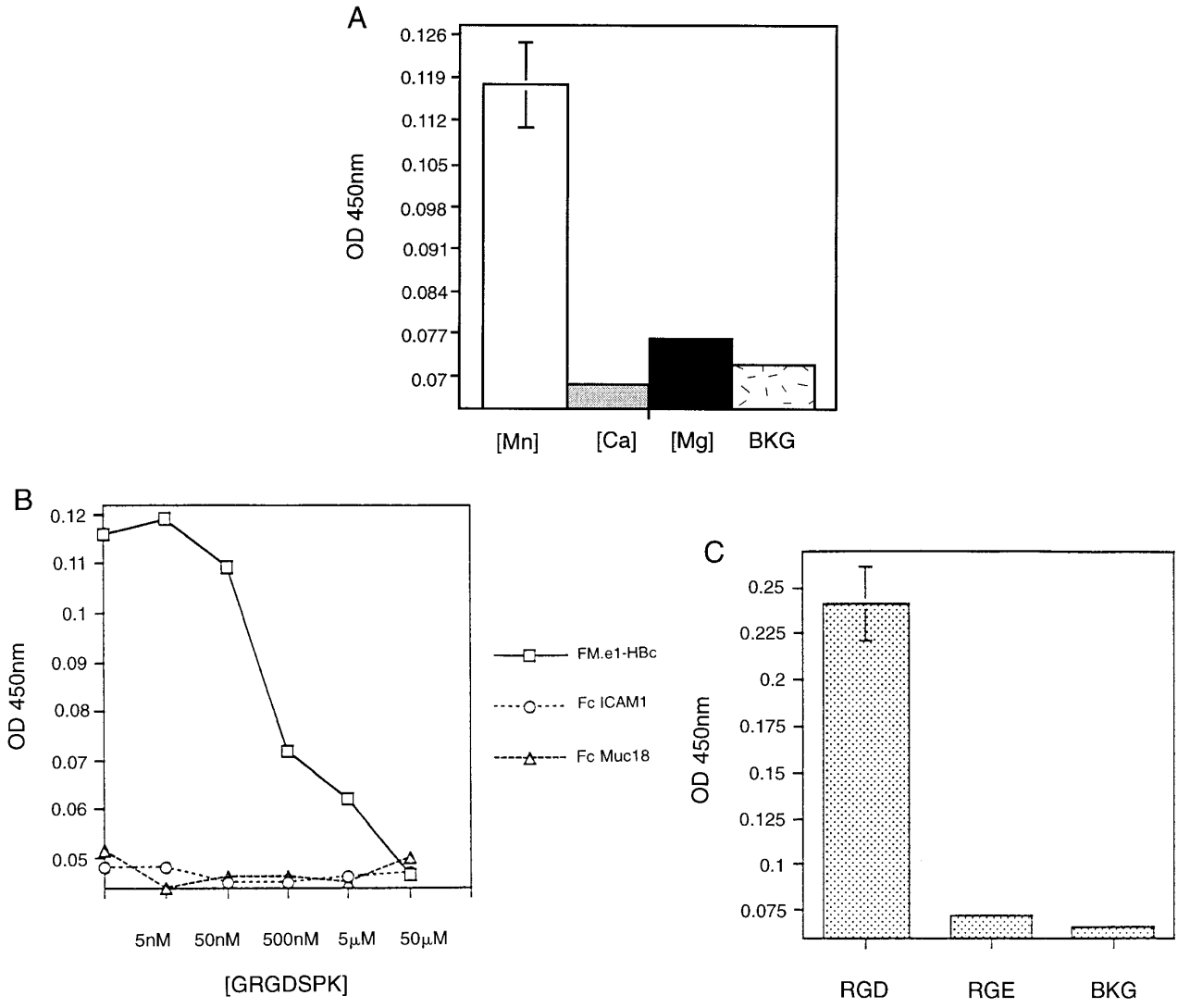


FIG. 3. Divalent cation dependence, peptide inhibition, and RGE mutant ELISA. FM.e1-HBc, mutant (RGE) FM.e1-HBc particles, and control proteins were coated on plates and integrin binding was measured as described under Materials and Methods. (A) Integrin $\alpha_v\beta_3$ was diluted in TBS or in TBS with 1 mM $MnCl_2$, $MgCl_2$, or $CaCl_2$ (added to the wells in 1-$\mu$l aliquots). Each experiment was done in quadruplicate and average values, including the background binding of FM.e1-HBc particles and Fc-Muc18 protein, are shown here (where error bars are not shown the standard deviation was less than 10% of the mean). (B) Peptide competition was performed by adding increasing concentrations (log range) of the peptide GRGDSPK to microtiter wells in 1-$\mu$l aliquots. Fc-ICAM1 and Fc-Muc18 were used as controls. The peptide concentration shown on the *x* axis is the final concentration in the well. This experiment was done in triplicate and average values are shown here (where error bars are not shown the standard deviation was less than 10% of the mean). (C) An RGE mutant of FM.e1-HBc particles was purified and used for solid-phase ELISA. The wells were coated in quadruplicate with FM.e1-HBc, mutant FM.e1-HBc, and a control protein, Fc-Muc18, and binding of integrin $\alpha_v\beta_3$ was performed as previously. RGD-containing FM.e1-HBc bound significantly to this integrin but the RGE mutant and the control protein Fc-Muc18 did not (where error bars are not shown the standard deviation was less than 10% of the mean).

an integrin which has been shown to act as a receptor for FMDV (Berinstein *et al.,* 1995). This binding has been studied in solid-phase ELISA assays as well as by electron microscopy. The data indicate that FM.e1-HBc/$\alpha_v\beta_3$ binding is divalent cation dependent, can be competed with fibronectin and vitronectin, and is inhibited by an RGD-containing peptide. The FM.e1-HBc/$\alpha_v\beta_3$ binding is dependent on the RGD sequence within the $\beta$G–$\beta$H loop as mutant particles in which the sequence was changed to RGE did not bind; binding is also dependent on $Mn^{2+}$ but not $Ca^{2+}$ or $Mg^{2+}$ cations. These results suggest that the chimeric particles mimic natural integrin/ligand interactions and the system should be valuable for addressing avidity and amino acid sequence differences between various pairs of integrin/ligand interactions.

Binding between FM.e1-HBc particles and $\alpha_v\beta_3$ is based on the inserted RGD-containing loop of FMDV. Clearly, this suggests that when the *R-G-D* sequence of residues is presented in an appropriate conformation, it can mimic natural ligands of integrins (one such integrin

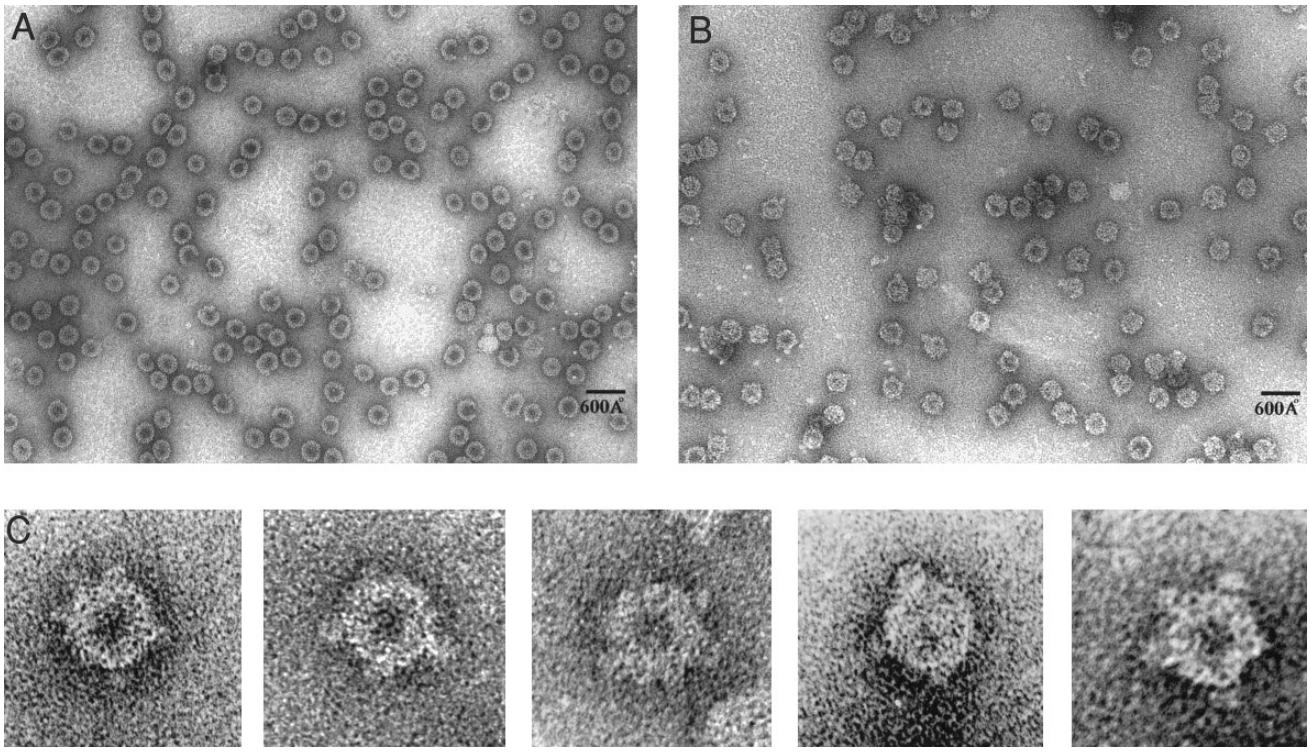


FIG. 4. Electron microscopy of FM.e1-HBc/$\alpha_v\beta_3$ complex. Samples were prepared as described under Materials and Methods. Bar represents ~600 Å. Electron micrograph of negatively stained sample of (A) FM.e1-HBc particles without any added $\alpha_v\beta_3$ and (B) with added $\alpha_v\beta_3$. (C) Different views of various FM.e1-HBc particles decorated with $\alpha_v\beta_3$ molecules.

being $\alpha_v\beta_3$) and so bind to cell surfaces. Several integrins are capable of binding to RGD-containing proteins (which are ubiquitous in eukaryotic cells) including $\alpha_{IIb}\beta_3$ and most of $\beta_1$ integrins (Hynes, 1992). Potentially, several RGD-binding integrins may therefore be able to bind to such mimetics. Therefore, "loop grafting," in which RGD-containing loops are inserted into nonadhesive proteins, may confer tissue-binding properties on a variety of biological entities (Maeda *et al.,* 1989; Smith *et al.,* 1995; Yamada *et al.,* 1995). The extent to which specific integrins can be targeted by altering the context of the RGD motif remains to be determined, but if this can be achieved it will bring a new dimension to the potential use of this technique for tissue-specific targeting.

The observation by EM of multiple $\alpha_v\beta_3$ molecules bound per particle indicates that there are no major conformational changes in the FM.e1-HBc upon the binding of the first $\alpha_v\beta_3$ molecule that may inhibit binding of other $\alpha_v\beta_3$ molecules. In the physiological context, FMDV or other RGD-containing infectious agents may bind to $\alpha_v\beta_3$ and engage multiple receptors from the same cell or different cells to produce an "*in vivo*" decoration. This may provide a "zippering mechanism" for internalization of the virus, and perhaps provide some seclusion from antibody detection or simply increase the likelihood of penetration into the cell by increasing the avidity of the interaction. The multiple receptor engagement per particle is likely to reflect a favored mode of virus/integrin interactions over the alternate possibility of virus/single molecule $\alpha_v\beta_3$ binding.

A variety of infectious agents use the RGD sequence to engage cellular receptors for infection, including (a) echovirus 22 which has an RGD motif in its VP1 protein and is inhibited by RGD-containing peptides (Hyypia *et al.,* 1992; Stanway *et al.,* 1994), (b) adenovirus which recognizes $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins by RGD-dependent mechanisms to aid infection (Defer *et al.,* 1990; Belin and Boulanger, 1993; Wickham *et al.,* 1993; Nemerow *et al.,* 1994), (c) the Lyme disease infectious agent, *Borrelia burgdorferi,* which mediates binding to platelet receptor integrin $\alpha_{IIb}\beta_3$ by using an RGD motif (Coburn *et al.,* 1993), and (d) the coxsackievirus A9 which uses its RGD motif to infect cells via $\alpha_v\beta_3$ (Roivainen *et al.,* 1994). In the case of FMDV, the critical role of the RGD loop of VP1 protein in FMDV infection is well established, as mutation of the RGD sequence in this loop abrogates virus attachment (Rieder *et al.,* 1994a,b). Recently, it has been conclusively shown that $\alpha_v\beta_3$ binding to FMDV occurs via the RGD sequence (Jackson *et al.,* 1997). However, in some cases the RGD "password" can be bypassed. Thus, FMDV complexed with antibodies can be infectious to cells via an antibody-dependent pathway in which the Fc receptors are used for attachment to cells (Mason *et al.,* 1993; Baxt and Mason, 1995), and coxsackievirus A9 remains

infectious when shorn of its RGD motif, entering cells via an alternate pathway (Hughes *et al.,* 1995).

It is possible that the RGD motif on a ligand constitutes a site of "primary interaction" with $\alpha_v\beta_3$, and thereby confers general specificity. Additional specificity may be conferred by other as yet undetermined contiguous or noncontiguous region(s) of the various ligands of $\alpha_v\beta_3$ which may constitute the site(s) for "secondary interaction." In this scenario, $\alpha_v\beta_3$ can differentiate between its various ligands containing the RGD motif subsequent to the primary interaction event (a) by making secondary interactions, (b) by the effects of various divalent cations which may modulate various conformational states of $\alpha_v\beta_3$, (c) by interacting with other moelcules, or (d) by a combination of these possibilities. If there is no region(s) in RGD-containing ligands which confers additional specificity for secondary interactions then $\alpha_v\beta_3$ may be a generic integrin receptor on the cell surface. Such a promiscuous receptor may demand nothing but a well-presented RGD loop for binding. The structural/biochemical basis underlying the interaction of $\alpha_v\beta_3$ with non-RGD-containing ligands like CD31 will be critical in establishing whether $\alpha_v\beta_3$ is such a generic integrin receptor. Future studies will focus on comparative analyses of adhesive interactions in the context of $\alpha_v\beta_3$-RGD-containing ligands versus $\alpha_v\beta_3$-CD31.

The biochemical and electron microscopic analyses presented pave the way to address the kinetic, thermodynamic, and mechanistic aspects of $\alpha_v\beta_3$-ligand (natural or synthetic) interactions. In addition, comparative studies with a range of integrins and viral sequences will help delineate rules for virus–integrin interactions. The data presented, and recent corroborating data (Berinstein *et al.,* 1995), strongly suggest that the bovine homologue of human $\alpha_v\beta_3$ is a cellular receptor for FMDV (type O). The decoration of FM.e1-HBc particles with individual $\alpha_v\beta_3$ molecules represents a significant advance, providing a model system for detailed structural/functional analysis of this physiological interaction. To our knowledge this is the first EM visualization of a virus–integrin complex, and it opens the door to the tantalizing prospect of cocrystallization of this and related complexes.

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