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Chimeric Hepatitis B Virus Core Particles as Probes for Studying Peptide-Integrin Interactions

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An RGD-containing epitope from the foot-and-mouth disease virus (FMDV) VP1 protein was inserted into the e1 loop of the hepatitis B virus core (HBc) protein. This chimeric protein was expressed at high levels in *Escherichia coli* and spontaneously assembled into virus-like particles which could be readily purified. These fusion particles elicited high levels of both enzyme-linked immunosorbent assay- and FMDV-neutralizing antibodies in guinea pigs. The chimeric particles bound specifically to cultured eukaryotic cells. Mutant particles carrying the tripeptide sequence RGE in place of RGD and the use of a competitive peptide, GRGDS, confirmed the critical involvement of the RGD sequence in this binding. The chimeric particles also bound to purified integrins, and inhibition by chain-specific anti-integrin monoclonal antibodies implicated $\alpha_5\beta_1$ as a candidate cell receptor for both the chimeric particle and FMDV. Some serotypes of FMDV bound to β_1 integrins in solid-phase assays, and the chimeric particles competed with FMDV for binding to susceptible eukaryotic cells. Thus, HBc particles may provide a simple, general system for exploring the interactions of specific peptide sequences with cellular receptors.

The foot-and-mouth disease viruses (FMDVs) make up the aphthovirus genus of the family *Picornaviridae*. The virus particles contain a single-stranded RNA genome within an icosahedral capsid consisting of 60 copies of each of four proteins, VP1 to VP4. The particle shell is made up from the three larger structural proteins, VP1 to VP3 (molecular weights, ca. 24,000), while the smaller VP4 (molecular weight, ca. 8,000) is located internally.

Several lines of evidence have demonstrated that a dominant neutralizing epitope of FMDV is located within a loop linking the βG and βH strands of the β barrel core structure of VP1 comprising amino acids 134 to 159. This loop (the G-H loop) is exposed as a highly mobile feature at the surface of the virus particle (1), and its immunogenicity can be mimicked by short (20- to 30-amino-acid) synthetic peptides that are able to elicit high levels of virus-neutralizing antibody and provide protective immunity in laboratory animals and, to lesser extents, in pigs and cattle, the target species for vaccination (5, 13, 20). The amino acid sequence of the loop shows extensive variation between different serotypes and subtypes of the virus, as would be expected for an immunodominant component of an antigenically variable virus.

In addition to its importance as a major immunogenic feature, the G-H loop has also been implicated in the binding of the virus to a cell receptor(s). A highly conserved amino acid triplet, RGD, is located within the loop and appears to be critically involved in the process of receptor binding. Short synthetic peptides that include this sequence can competitively inhibit virus binding to cells (3, 14, 28), and mutation of the sequence can also abrogate virus attachment (19, 23). Since RGD is the signature of ligands bound by several members of the integrin family of cell receptors, it has been suggested that the receptors used by FMDVs belong to this family. Further support for this proposal is provided by the structure of the RGD in the loop, which is in the conformation seen in proteins which are known to bind integrins (15), and by the critical involvement of divalent cations in virus attachment.

It is unlikely that the RGD triplet alone dictates the specificity of virus binding, since competition binding experiments suggested that viruses of different serotypes have only partially overlapping receptor specificities (26). Also, proteolytic removal of the C-terminal portion of VP1, which is adjacent to the G-H loop at the surface of the virus particle, under conditions that leave the G-H loop intact has been shown to interfere with virus binding (14, 27).

In view of these observations, it was of interest to determine whether the G-H loop sequence alone contains the features responsible for the binding of FMDV to cells. To examine this question, we have inserted the FMDV sequence into the e1 loop of the hepatitis B core (HBc) antigen (Ag) protein, since at this location it would be expected to be exposed at the surface of the protein and to be presented in multiple copies on assembled recombinant particles (FM-HBc particles) as with FMDV particles (2). Furthermore, the constraints imposed by inserting the sequence within a loop of the assembled FM-HBc protein are likely to result in it adopting a more native conformation, as has been shown with other viral epitopes (6, 10).

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In earlier studies we had examined the immunogenic properties of the recombinant protein in which the FMDV VP1 140 to 160 sequence was fused to the N terminus of the HBc Ag (here termed FM.N-HBc) (11). Although attempts to express this material in *Escherichia coli* were unsuccessful, the protein could be produced in mammalian cells with recombinant vaccinia virus. It assembled into HBc particles that could readily be purified and were found to be potent immunogens. In this study we have shown that HBc protein in which the sequence 141 to 160 of VP1 has been inserted within the e1 loop (FM.e1-HBc) can be expressed to high levels in *E. coli*, in which it assembles into isometric particles. These can elicit high levels of virus-neutralizing and -protective antibodies. Furthermore, the recombinant particles have potent cell-binding activity that is dependent, in part, on the tripeptide sequence RGD and are able to compete with the binding of intact FMDV particles. They also bind to isolated integrins in solid-phase assays. The approaches used in this study suggest a simple model system for the investigation of receptor-ligand interactions.

MATERIALS AND METHODS

Construction of recombinant plasmid pFM.e1. All recombinant DNA experiments were done with *E. coli* XL-1 Blue and were based on plasmid pPV-Nhe described previously (6). An 84-bp double-stranded DNA oligonucleotide flanked with *Nhe*I restriction sites and corresponding to the hypervariable region (amino acids 135 to 160) of VP1 of FMDV serotype O1 was generated as two complementary double-stranded oligonucleotides, annealed, digested with *Nhe*I, and cloned into the corresponding site of pPV-Nhe. A plasmid containing the insert in the correct orientation was isolated and termed pFM.e1. Plasmid pFM(E).e1 was constructed as described above with synthetic oligonucleotides that altered the coding potential for the sequence RGD in the FMDV insert to RGE.

Induction and purification of fusion protein. Bacteria harboring either pPV-Nhe or pFM.e1 plasmids were grown overnight in L-ampicillin medium to high cell density and diluted with fresh broth (1:10) the following day. Expression was routinely induced by the immediate addition of isopropyl-β-D-thiogalactoside (IPTG; final concentration, 60 μg/ml) when the culture reached an A_{650} of 1.0 and was allowed to replicate for a further 6 to 8 h at 37°C. Cells were then harvested, and HBc particles (PV-1-HBc and FM.e1-HBc, respectively) were purified as described previously (6). The concentration of HBc preparations was determined with a bicinchoninic acid protein quantification kit (Pierce).

ELISA. HBc preparations were used to coat 96-well enzyme-linked immunosorbent assay (ELISA) plates (Costar enzyme immunoassay plates) before incubation with a rabbit anti-HBc Ag antibody (DAKO B586). Bound antibody was measured by the addition of horseradish peroxidase-conjugated swine anti-rabbit immunoglobulins (DAKO P217) and an enzyme substrate (0.04% *o*-phenylenediamine and 0.004% hydrogen peroxide in 0.1 M phosphate–0.05 M citrate buffer). Antipeptide and anti-HBc Ag activities in animal serum samples were measured by modifications of the indirect or double-antibody sandwich ELISA method (29) as described in reference 6.

Electron microscopy. HBc particle preparations were spotted onto carbon-coated grids, negatively stained with uranyl acetate, and visualized in a Hitachi 600 transmission electron microscope with an accelerating voltage of 80 kV.

Animal immunization. Female Dunkin Hartley guinea pigs were injected intramuscularly with emulsions of FM.e1-HBc particles emulsified with Freund's incomplete adjuvant. Animals received 2 or 20 μg of FM.e1-HBc per injection and were given booster injections with the same preparations at 70 days. Samples of blood were collected and analyzed at intervals as detailed in Results.

Cell lines. The following established cell lines were purchased from ICN Flow: HEp-2, BHK-21, PK(15), HeLa, Vero, and LM. All were grown in the company's recommended culture medium at 37°C in a humidified atmosphere containing 5% CO_2.

Flow cytometry. A 24-well plate (Costar) was coated with 500 μl of the target cell line per well at a density of 10^5 cells per well and incubated overnight. The culture medium was replaced with 200 μl of solution 1 (RPMI 1640, 0.4% bovine serum albumin [BSA], 20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid]) and 100 μl of HBc preparation at concentrations of 100, 50, 10, and 0 μg/ml. The cells were incubated for 5 min at 37°C and then for 2 h at room temperature. The cells were washed once with 200 μl of solution 1, incubated for 5 min at room temperature, and then washed four times with 200 μl of phosphate-buffered saline (PBS). DAKO B586 anti-HBc Ag antibody at a 1/100 dilution was added at 200 μl per well and incubated for 1 h at 37°C. The wells were then washed three times with solution 2 (RPMI 1640, 20 mM HEPES) and once with PBS. Fluorescein isothiocyanate-conjugated swine anti-rabbit immunoglobulin antibody (DAKO F205) was diluted 1/1,000 and added at 200 μl per well, and the plate was incubated for 30 min at 37°C. The wells were then washed three times with solution 2 and once with PBS. Cell dissociation fluid (500 μl; Sigma) was added, and the plate was incubated for 10 min at 37°C. The cells were washed from the plastic into FACSort tubes and transferred to a FACSort machine (Becton Dickinson).

Counting 10^4 cells per run, the fluorescence of samples containing the HBc preparation was measured against a control sample containing cells and antibody but incubated in the absence of HBc. The percent fluorescence above background was calculated as follows. The fluorescence of cells that had been incubated with antibody but no HBc was measured. The threshold was set so that the number of cells with a fluorescence intensity above this limit was <1%. Samples that had been incubated with HBc were then run with the threshold unaltered. The number of cells with a level of fluorescence now above this limit was determined and expressed as a percentage of the total number of cells sampled.

Cell-binding assay. A 96-well (Costar enzyme immunoassay-radioimmunoassay) plate was coated with a nine-step doubling dilution series of HBc preparation starting at 100 μg/ml and incubated overnight. The plate was washed twice with PBS and then blocked with 100 μl of 3% BSA in PBS per well for 1 h at 37°C. The plate was washed three times with PBS before the addition of 50 μl of HEp-2 cells per well at a concentration of 6×10^5 cells per ml. The plate was reincubated at 37°C for a further 1.5 h. All wells of the plate were washed carefully with PBS until no bound cells remained in the control wells containing PBS alone (approximately five times). All remaining cells on the plate were fixed with 0.2% glutaraldehyde for 10 min at room temperature. The fixed cells were washed three times with PBS and stained with 0.2% crystal violet for 1 h at room temperature. Unadsorbed stain was washed from the plate with PBS, and the cells were lysed with 1% sodium dodecyl sulfate (SDS) for 10 min. The plate was transferred to a microplate reader (Ceres 900; Anachem), and the A_{562} was read.

FMDV. Viruses of serotypes O (strain BFS 1860), A (A12), SAT1 (Sudan 8/69), and SAT2 (Rho 1/48 and Ken 183/74) were grown in BHK-21 cell monolayers, radiolabelled where appropriate with [^{35}S]methionine, and purified as described previously (7).

Neutralization assay. A neutralization assay was performed as previously described (25). Antibody titers are expressed as $\log_{10}$ of the reciprocal serum dilution giving 50% neutralization of 100 tissue culture infective doses of virus.

FMDV competition binding assay. FM.e1-HBc in 2 ml of Eagle's medium was added to each 25-cm^2 BHK-21 cell monolayer and incubated at 37°C for 1 h before 100 μl of [^{35}S]methionine-labelled purified FMDV was added. At various time intervals, 50 μl of the medium were removed and counted in a scintillation counter (model LS 5000; Beckman, Palo Alto, Calif.). The reduction in counts was converted to represent the percentage of labelled virus attached to cells.

Solid-phase receptor-ligand binding assay. Lymphocyte β_1 integrins were prepared as previously described (17). Integrin $\alpha_5\beta_1$ was found by ELISA to be a major component of this mixture. Solid-phase ligand-receptor binding was performed by a modification of a method previously described (8). Purified integrins (at a concentration of ~500 μg/ml) were diluted 1/100 with PBS containing divalent cations, and 100-μl aliquots were added to the wells of a 96-well ELISA plate (Dynatech Immulon). Plates were incubated overnight at 4°C, and the wells were then blocked for 3 h at room temperature with 200 μl of 5% (wt/vol) BSA–150 mM NaCl–0.05% (wt/vol) NaN_3–25 mM Tris-HCl (pH 7.4). Wells were washed twice with 200 μl of buffer A (150 mM NaCl, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 25 mM Tris-HCl [pH 7.4], 1 mg of BSA per ml), and 100-μl aliquots of HBc preparation (0.1 to 10 μg/ml) diluted in buffer A were added, with or without competitor antibodies or peptide. The plates were then incubated at 30°C. After 3 h, excess ligands were removed and the wells were washed three times with buffer A. Bound ligand was quantified with DAKO B586 anti-HBc Ag antibody or anti-HBc Ag guinea pig serum and then with the relevant horseradish peroxidase antispecies conjugate. Wells were then washed three times with buffer A. Where the DAKO primary antibody was used, color was developed with ABTS (Sigma) and the A_{410} was read. Where the guinea pig antiserum was used, color was developed with OPD-H_2O_2, the development was stopped by the addition of 1 M H_2SO_4, and A_{492} was read. The level of nonspecific binding was measured by determining the level of ligand binding to wells coated with BSA alone (for the data shown in Fig. 8). These values were subtracted from the corresponding values for receptor-coated wells.

RESULTS

Expression and purification of HBc particles. In contrast to earlier studies involving recombinant HBc constructs in which the FMDV VP1 140 to 160 sequence was fused to the N terminus of the HBc Ag protein, the e1 loop insertion construct FM.e1-HBc protein was not toxic to *E. coli* and could be expressed to high levels, following induction with IPTG. The expressed protein retained the ability to assemble into 27-nm particles which were soluble and could readily be purified from bacterial extracts by a combination of differential and sucrose gradient centrifugation, as previously described for other chimeric constructs (10). The purified material reacted with anti-HBc Ag antisera in ELISA, and SDS-polyacrylamide gel elec-

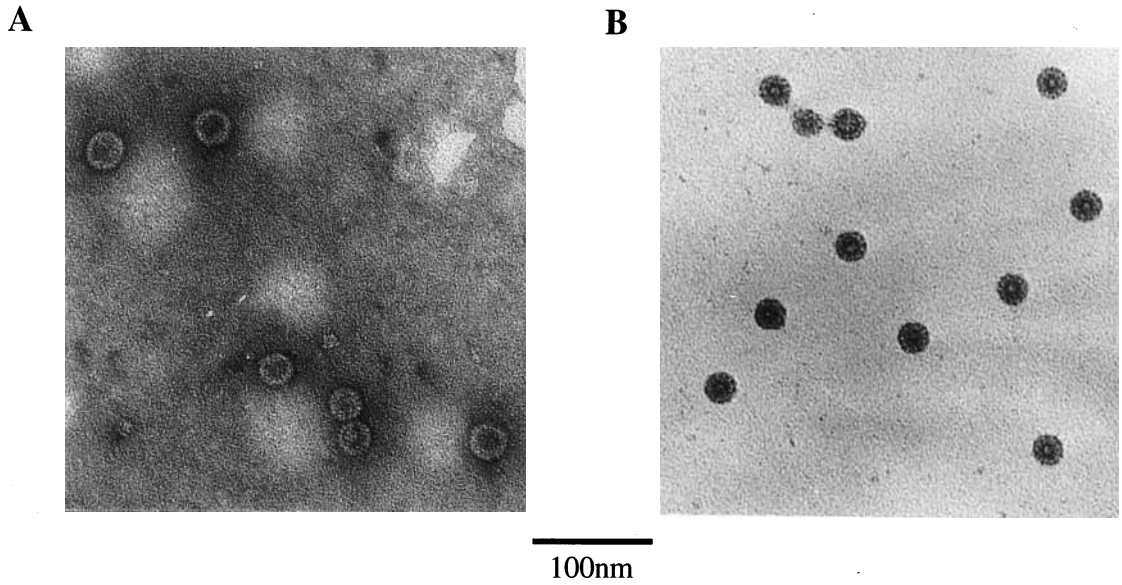

FIG. 1. Electron micrograph of HBc particles. (A) FM.e1-HBc particles. (B) PV-1-HBc particles.

trophoresis showed a single band with the predicted mobility (results not shown). Examination by electron microscopy showed the material to be composed of isometric particles of typical HBc Ag morphology (Fig. 1). HBc particles which lacked the FMDV insert (PV-1-HBc) were prepared in the same way and displayed similar properties.

Immunogenic properties of FM.e1-HBc particles. High levels of both anti-FMDV peptide and virus-neutralizing antibodies were induced in guinea pigs injected with FM.e1-HBc particle preparations emulsified in Freund's incomplete adjuvant (Table 1). Similar titers were obtained following immunization with either 2 or 20 μg of recombinant particles, and these titers were not significantly increased following booster injections of the same amounts of Ag after 70 days. Thus, it would appear that a single dose of 2 μg of the recombinant material was sufficient for maximal stimulation of the immune response. It also indicates that the inserted peptide accurately reproduces the site present in the authentic virus.

Binding of FM.e1-HBc particles to cells and inhibition with anti-141-to-160 antibody. Insertion of the FMDV VP1 G-H loop sequence into the HBc protein sequence was found to greatly increase the ability of the recombinant particles to attach to a range of cell lines originating from different mammalian species (Fig. 2), suggesting that binding of FM.e1-HBc to cells is predominantly mediated through the FMDV sequence contained within the e1 loop. To examine this possibility, FM.e1-HBc particles were incubated with HEp-2 cell monolayers in the presence of an anti-VP1 141 to 160 peptide antibody at varying HBc/antibody ratios (Fig. 3). The antibody had a clear inhibitory effect, but its inability to completely abrogate binding at the highest particle concentration is probably indicative of the multivalent nature of the HBc particle, because each has potentially 180 or 240 sites of attachment through the e1 loop region, depending on the assembled morphology (12).

The effect of RGD → RGE mutagenesis on cell binding. Since the binding of FM.e1-HBc to HEp-2 cells is mediated through the FMDV sequence contained within the e1 loop, we examined the significance of the RGD sequence within this region for cell binding. The ability of FM(E).e1-HBc cores containing RGE instead of RGD to promote cell binding was compared with those of FM.e1-HBc and PV-1-HBc. Such RGD-to-RGE mutations have been shown to abrogate the binding of the ligand to a variety of integrins (9, 22). Figure 4 shows that the single-amino-acid substitution in our experiments resulted in a reduction of the cell binding ability to levels

TABLE 1. Antibody response to FM.e1-HBc particles in guinea pigs

Days after primary inoculation	Response to FM.e1-HBc particles[a] with:					
	20 μg of antibody:			2 μg of antibody:		
	Anti-HBc	Antipeptide	Neutralizing	Anti-HBc	Antipeptide	Neutralizing
0	<1.0	<1.0	<0.6	<1.0	<1.0	<0.6
14	1.3	2.5	1.8	1.5	2.1	1.4
28	3.6	3.7	2.9	3.1	3.4	2.1
42	3.6	4.3	3.0	3.6	3.8	2.8
70 (booster injection)	4.2	4.5	3.3	3.9	4.3	3.3
77	4.4	4.8	3.0	4.2	4.2	3.2
84			3.6			3.6
98			3.8			3.2

[a] Expressed as the log reciprocal dilution giving 50% activity by ELISA or virus neutralization assay.

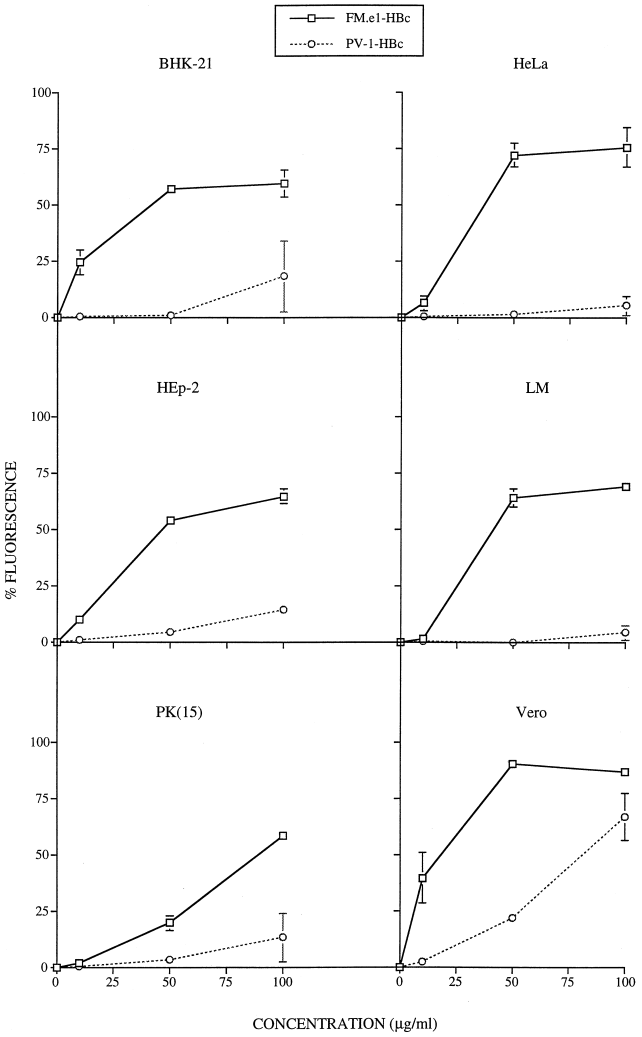

FIG. 2. Binding of FM.e1-HBc and PV-1-HBc particles to a variety of cell lines. The degrees of binding are represented by the percentages of fluorescence above background with a range of concentrations of HBc particles, determined by flow cytometry as described in Materials and Methods. Error bars show the standard errors of the mean.

below that of the background at all concentrations up to and including 100 μg/ml.

Inhibition of FMDV-cell attachment by FM.e1-HBc. We were interested in the capacity of FM.e1-HBc to inhibit the binding of FMDV to cells in vitro, since it has been shown that RGD-containing peptides have this capability and that the RGD sequence at positions 145 to 147 of the hypervariable region of VP1 appears to contribute to the cell attachment site for BHK cells (3, 14, 23, 28). Figure 5A and B show the results with FMDV serotypes A12 and O1K, respectively. For both serotypes, FM.e1-HBc was capable of competing with FMDV for attachment to BHK cells. The inhibition of FMDV binding was apparent after 10 min of incubation and was seen with 10 μg of FM.e1-HBc per ml.

The binding of HBc to integrins. Since many integrins have been shown to interact with extracellular matrix proteins containing the sequence RGD, we examined the ability of PV-1-HBc, FM(E).e1-HBc, and FM.e1-HBc to bind integrins. Figure 6 shows the binding of PV-1-HBc and FM.e1-HBc to β_1 integrins in solid phase. There was no significant binding in the absence of integrin; however, when β_1 integrins were present, FM.e1-HBc particles bound with high affinity. As anticipated, PV-1-HBc did not bind to the integrin-coated plastic.

The ability of FM.e1-HBc to bind to β_1 integrins was further examined in the presence of EDTA, peptide competitor, or monoclonal antibodies (MAbs) specific to integrin alpha or beta chains (Fig. 7). All binding of FM.e1-HBc was lost in the presence of 5 mM EDTA. Since binding of integrins to their ligands is dependent on divalent cations, this effect of EDTA provides further evidence that binding is exclusively mediated through integrins. Binding was inhibited by a peptide with sequence GRGDS at 100 μg/ml, further indicating that the RGD motif of the FM.e1-HBc is largely responsible for receptor binding. Binding was significantly inhibited by MAbs specific to integrin chains β_1 (MAb13) and α_5 (MAb16) but not to α_4 (HP2/1). Furthermore, since MAb11 was inactive, the inhibition of binding by monoclonal antibodies to the α_5 chain appeared to be significant only when the MAb had an antifunctional capacity for mammalian cells, suggesting that FM.e1-HBc binds at, or very close to, the characterized functional domain of the α_5 chain.

The binding of FMDV to integrins. Since FM.e1-HBc particles were found to bind β_1 integrins in solid phase, we exam-

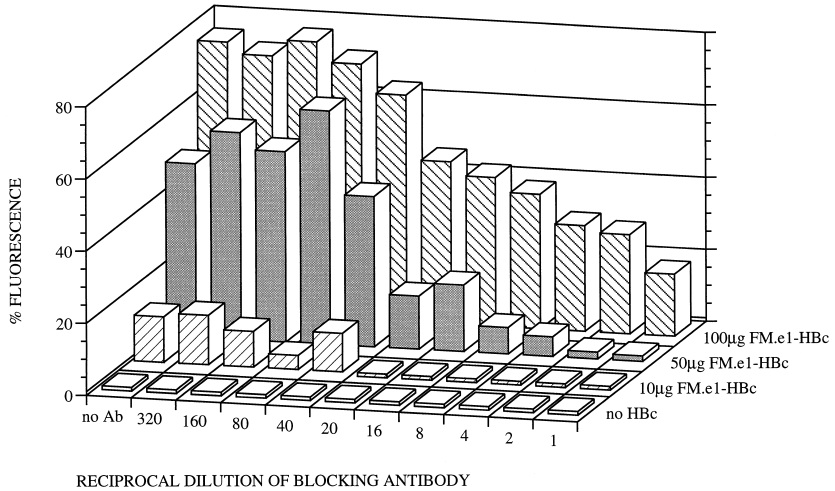

FIG. 3. Inhibition of binding of FM.e1-HBc particles to HEp-2 cells by anti-VP1 141 to 160 peptide antibody. Inhibition is represented by decreases in the percentages of fluorescence above background with a range of FM.e1-HBc concentrations versus reciprocal dilutions of the blocking antibody to VP1 141 to 160 peptide.

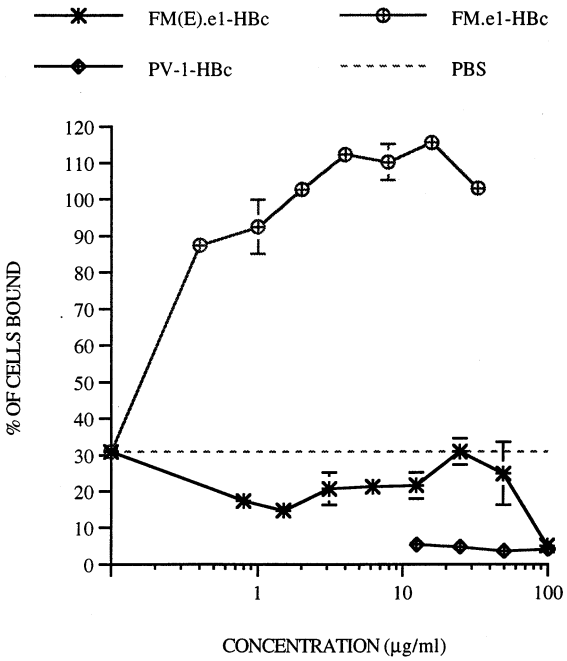

FIG. 4. Effect of HBc mutagenesis on the binding of HEp-2 cells to HBc particles in solid phase. FM(E).e1-HBc proteins were generated as described in Materials and Methods and differ from FM.e1-HBc only in that the aspartic acid (D) of the RGD sequence has been replaced by a glutamic acid (E). The degrees of cell binding are expressed as the percentages of HEp-2 cells bound to PV-1-HBc, FM.e1-HBc, and FM(E).e1-HBc particles in solid phase with a range of concentrations. The background level of cell binding was determined in PBS and is shown by the straight dashed line. Error bars show the standard errors of the mean.

ined whether FMDV would behave similarly, as suggested by the results shown in Fig. 5. The results reflected in Fig. 8 show the ability of FMDV of various serotypes to bind β_1 integrins in solid phase. All strains tested of the A and O serotypes showed no significant binding to the integrins. In addition, no binding was found with FMDV C Noville (data not shown). Viruses belonging to the SAT2 serotype agglutinate erythrocytes and may differ from other serotypes in exhibiting a degree of nonspecific binding. However, two strains of the SAT2 serotype and one strain of the SAT1 serotype were found to bind to the integrin preparation significantly above background levels. For SAT1 Sudan 8/69, this was apparent only at a virus concentration of 10 μg/ml.

DISCUSSION

We have shown that insertion of an FMDV epitope within the e1 loop of HBc protein allowed the high-level expression in *E. coli* of a chimeric protein (FM.e1-HBc) which assembled into virus-like particles and could be readily purified. These fusion particles were extremely immunogenic and elicited high levels of both ELISA and virus-neutralizing antibodies in guinea pigs. Further, the chimeric particles were able to bind specifically to cultured eukaryotic cells and to purified integrins and to compete with infectious FMDV for binding to susceptible, cultured eukaryotic cells. Thus, HBc particles provide a simple general system for exploring the interaction of specific peptide sequences with cellular receptors.

Previously published experiments utilizing the insertion of FMDV peptides into HBc particles were directed at enhancing the immune response to these peptides. These experiments involved fusing FMDV-based peptide sequences to the N terminus of the HBc protein (11). Subsequent experiments with epitopes derived from other viruses showed that insertion of foreign peptides within the sequence of the HBc protein, at a position predicted to be part of a major immunodominant, surface-associated loop (the e1 loop) (6), increased their immunogenicity approximately 10-fold compared with that of N-terminal fusion (6). Moreover, in some cases, insertion of sequences at this position in the HBc protein was found to confer a conformational advantage on the fusion peptide, as evidenced by improved reactivity with antiviral MAbs (6). In addition to its importance as a major immunogenic feature, the VP1 G-H loop has been implicated in the binding of the virus to cell receptor(s). Taken together, these data prompted us to insert the VP1 G-H loop of FMDV into the e1 loop of HBc.

The FM.e1-HBc particles were easily purified from *E. coli* and were highly immunogenic. Although the sequence of the G-H loop of VP1 is highly variable, both in length and composition, between different isolates of the virus, it includes a highly conserved triplet, RGD. This sequence is critical for the binding of proteins to many members of the integrin family of cell receptors (24). Competitive binding studies using synthetic peptides (3, 14, 28) and directed mutagenesis of the virus via an infectious clone (19, 23) have shown that the RGD sequence is important for the binding of FMDV to its cell receptor(s). Expression of the VP1 G-H loop sequence at the surface of HBc particles conferred on them the ability to attach

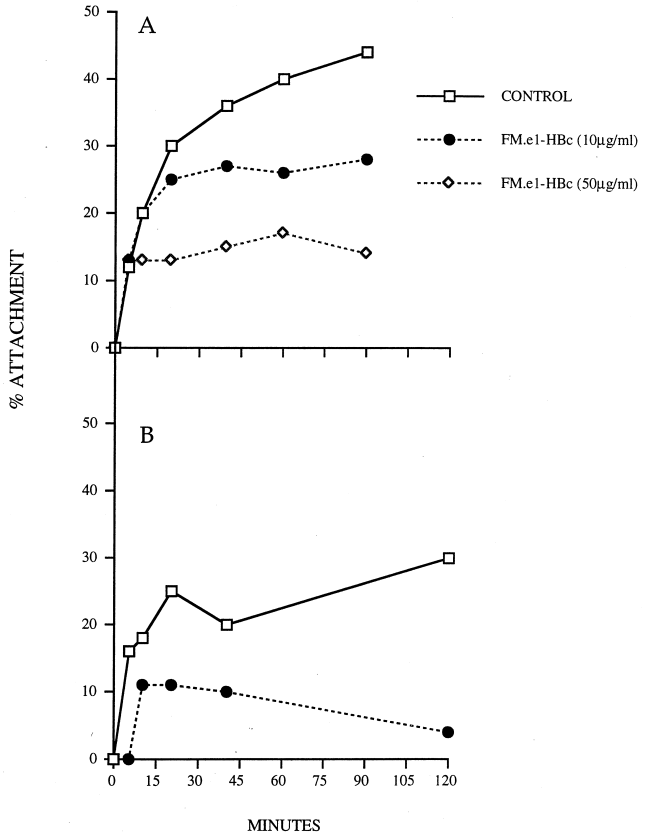

FIG. 5. Inhibition of the binding of FMDV to BHK cells by the presence of FM.e1-HBc particles. The degrees of attachment of FMDV serotype A12 (A) and FMDV serotype O1K (B) to BHK cells are expressed as percentages over time in the absence (control) or presence of FM.e1-HBc particles at the concentrations shown.

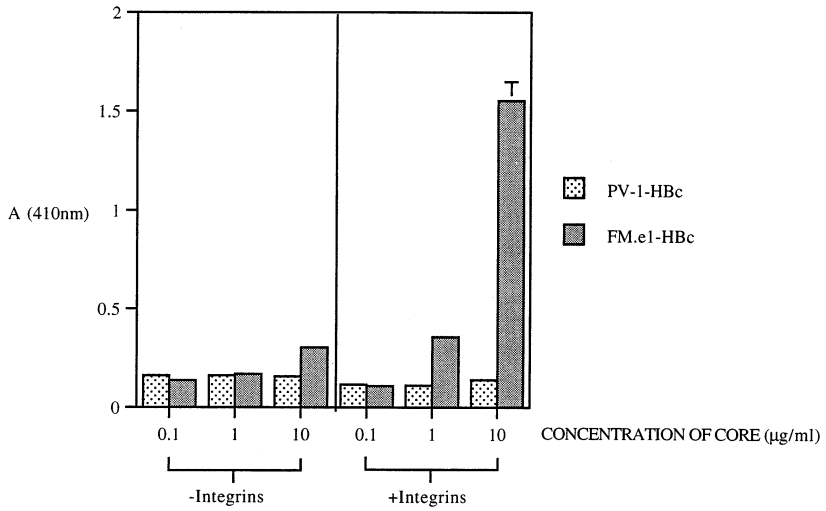

FIG. 6. Binding of HBc particles to β_1 integrins in solid phase. The degrees of binding are expressed as A_{410} as described in Materials and Methods. The binding of FM.e1-HBc and PV-1-HBc particles at three concentrations was examined both in the presence (+) and absence (−) of integrins. The error bar shows the standard error of the mean.

to a wide range of cells in culture, lending further weight to the argument that it contains an important component of the cell attachment site of the virus. Although the range of cells to which FMDVs can attach has not been studied exhaustively, they are known to bind to BHK-21 and PK(15) cells, since these can support growth of the viruses, and, to a lesser extent, to LLC-MK2, CHO, and HeLa cells (4).

Antisera against synthetic peptides representing the VP1 G-H loop sequence of FMDV effectively neutralize infectivity by preventing virus particles from attaching to susceptible cells (3, 14). However, antibody-complexed FMDV has been found to be infectious for cells bearing Fc receptors, showing that antibody-complexed virus is potentially infectious if it can attach to cells (18). These antipeptide antibodies could also inhibit the attachment of the FM.e1-HBc particles to cells in culture, thus showing that their cell attachment properties were a direct consequence of the presence of the FMDV sequence. The FM.e1-HBc particles competitively inhibited the binding of FMDV to cells, as would be predicted if they attached to the same receptor. Furthermore, the core particles were approximately 1,000-fold more effective on a molar basis than short RGD-containing peptides in competing for virus binding to cells (14). Also, the conformation of the VP1 G-H loop expressed on HBc particles appears to better represent that found on the virus, since synthetic peptides comprising the complete G-H loop sequence had little or no activity in competition binding experiments. The apparently high avidity of binding of the FM.e1-HBc particles to cells is partly a consequence of the high density of expression of the FMDV sequence at their surfaces, since disruption of the particles into monomeric protein (10, 14, 16) dramatically reduced binding (data not shown).

The similarities between the cell-binding properties of FMDV and the FM.e1-HBc particles suggest that they bind to the same receptor(s), and the key involvement of the RGD triplet in this binding indicates this is an integrin. Indeed, the FM.e1-HBc particles were shown to bind to purified preparations of β_1 integrins bound to a plastic surface. As might be predicted from the known requirement for divalent cations in the interaction between integrins and their ligands, the binding of the FM.e1-HBc particles was inhibited in the presence of EDTA. The attachment of FMDV to cells is similarly known to require the presence of divalent cations. Again, the crucial involvement of the RGD triplet in binding to the immobilized purified integrins was implied from the ability of the short peptide GRGDS to compete for particle attachment.

Mutation of the RGD motif to RGE in an infectious clone of the virus has been shown to compromise the attachment properties such that infectivity can be demonstrated only when cell binding is mediated by a surrogate receptor, such as the Fc receptor (18). The consequences of the same mutation in the binding properties of FM(E).e1-HBc were assessed in a cell binding assay in which HBc particles were adsorbed onto a

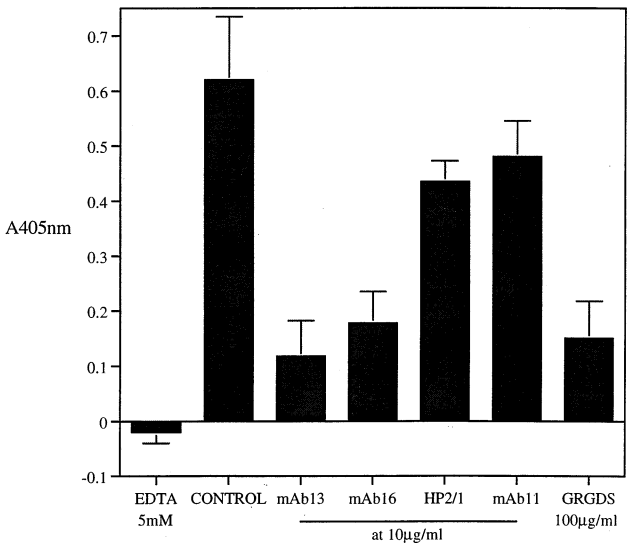

FIG. 7. Specificities of the binding of FM.e1-HBc particles to β_1 integrins in solid phase. The degrees of binding are expressed as A_{405} as described in Materials and Methods. The degrees of association were determined in the presence of 5 mM EDTA and the pentapeptide GRGDS. The specificities of association were determined with four MAbs with the following specificities: MAb13, anti-β_1; MAb16, anti-α_5, antifunctional; HP2/1, anti-α_4; MAb11, anti-α_5, not antifunctional. Error bars show the standard errors of the mean.

plastic surface and their function as a substrate for cell attachment was recorded as a measure of cell settling and flattening over time. By this assay, it was apparent that FM.e1-HBc particles could substitute for an extracellular matrix, since their presence on the plastic surface markedly increased the percentage of cells bound and indeed their rate of cell spreading (data not shown). This effect was lost, however, with the particles carrying the D-to-E mutation.

Initial experiments showed that the FM.e1-HBc particles could bind well to purified β_1 integrins immobilized by adsorption onto a plastic surface. In further studies using a limited repertoire of antifunctional MAbs specific for integrin subunits, it was found that α_5 and β_1 chains were important for the binding of FM.e1-HBc particles. Neither an antibody directed against α_4 nor one which recognized α_5 but did not inhibit its function had significant effects on binding. This is in apparent disagreement with the findings of Berinstein et al. (4), who found that the binding of FMDV serotype A12 to cells in culture was inhibited by polyclonal antisera to the vitronectin receptor ($\alpha_v\beta_{3/5}$), but not the fibronectin receptor ($\alpha_5\beta_1$), and by MAbs to α_v and β_3, but not to $\alpha_v\beta_5$. However, the proportional representation of the different species of integrins present in the purified preparations may be significantly different from that on cell surfaces: by ELISA, the integrin $\alpha_5\beta_1$ was found to comprise 20 to 30% of the lymphocyte β_1 integrin preparation (data not shown). Furthermore, we cannot comment on the ability of FM.e1-HBc particles to bind to β_3 integrins, since our purified preparations contained only β_1 molecules. It is possible that features on the virus surface may modulate receptor binding even if this is primarily directed by the sequence of the G-H loop. The reduction in virus binding seen when the C-terminal portion of VP1 is proteolytically removed while leaving the G-H loop intact would support this suggestion (14).

It is surprising that most strains and serotypes of the virus tested bound poorly, if at all, to the immobilized β_1 integrins, even though FM.e1-HBc particles bound effectively to the same preparations. It is possible that the preferred receptors for virus attachment were not represented in the integrin preparations used or that the FM.e1-HBc particles lack the fine specificity displayed by the virus and bind more promiscuously. The exception to this was viruses of serotype SAT2, which bound well. It is of interest that the SAT2 viruses differ from those of other serotypes in their ability to hemagglutinate erythrocytes. The poor binding of most viruses compared with that of the recombinant HBc particles may be due to the lower density of expression of the G-H loop on the virus. Alternatively, it is possible that the inherent integrin binding activity associated with the G-H loop sequence and structure is subtly modulated by its environment on the intact FMDV particle. Further study of the properties of recombinant particles, such as those described in this report, should help to resolve the essential features of the attachment of FMDV to cell surfaces.

Synthetic peptide vaccines based on the sequence of the immunodominant epitope of FMDV have been poorly effective in cattle, despite the vaccines' highly protective capacities in guinea pigs. It has been suggested that this poor level of effectiveness may result from differences between the helper T-cell responses induced by peptide or virus Ags (4). It will be interesting to determine whether the immune response in cattle to FM.e1-HBc particles resembles that to synthetic peptide or inactivated virus vaccines.

In conclusion, we have shown that chimeric HBc particles can readily be used to explore the cell-binding potential of peptide sequences. Core particles offer advantages over some other systems in that they can easily be purified and assayed with immunological tags. Any peptide sequence inserted into HBc particles will be present in multiple copies (at least 180 copies per particle), thus increasing valency and improving the sensitivity of interactions with receptors. Inserted sequences within the HBc gene may be mutagenized to explore the role of individual residues within the sequence, providing that adequate precautions are taken to eliminate nonspecific binding. Further, we are presently attempting to adapt the system so that randomized peptide sequences can be incorporated into core particles for the purpose of developing multivalent peptide libraries suitable for panning for adhesive sequences.

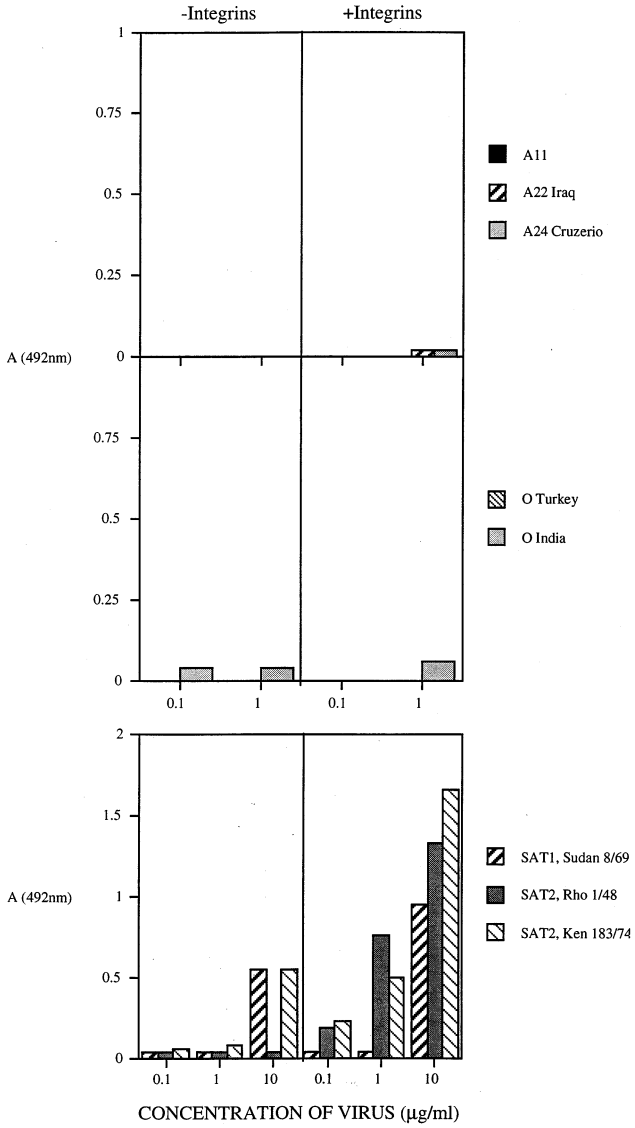

FIG. 8. Binding of a variety of FMDV serotypes to β_1 integrins in solid phase. The degrees of binding are expressed as A_{492} as described in Materials and Methods. The binding of FMDV serotypes at the concentrations shown was examined both in the presence (+) and absence (−) of integrins.

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