JCM
Journals.ASM.org

A Continuous Bovine Kidney Cell Line Constitutively Expressing Bovine $\alpha_V\beta_6$ Integrin Has Increased Susceptibility to Foot-and-Mouth Disease Virus

Michael LaRocco,[a] Peter W. Krug,[a] Ed Kramer,[b] Zaheer Ahmed,[a] Juan M. Pacheco,[a] Hernando Duque,[c] Barry Baxt,[a] Luis L. Rodriguez[a]

Foreign Animal Disease Research Unit, United States Department of Agriculture, Agricultural Research Service, Plum Island Animal Disease Center, Greenport, New York, USA[a]; The McConnell Group, Inc., Rockville, Maryland, USA[b]; Foreign Animal Disease Diagnostic Laboratory, United States Department of Agriculture, Animal and Plant Health Inspection Service, Plum Island Animal Disease Center, Greenport, New York, USA[c]

Foot-and-mouth disease (FMD) is a worldwide problem limiting the trade of animals and their products from affected countries. The rapid isolation, serotyping, and vaccine matching of FMD virus from disease outbreaks is critical for enabling the implementation of effective vaccination programs and to stop the spread of infection during outbreaks. Some primary cells have been shown to be highly susceptible to most strains of FMD virus (FMDV) but are difficult and expensive to prepare and maintain. Since the $\alpha_V\beta_6$ integrin is a principal receptor for FMDV, we transduced a bovine kidney cell line to stably express both the α_V and β_6 bovine integrin subunits. This stable cell line (LFBK-$\alpha_V\beta_6$) showed β_6 expression and enhanced susceptibility to FMDV infection for ≥100 cell passages. LFBK-$\alpha_V\beta_6$ cells were highly sensitive for detecting all serotypes of FMDV from experimentally infected animals, including the porcinophilic FMDV strain O/TAW/97. In comparison to other cell types that are currently used for virus isolation, LFBK-$\alpha_V\beta_6$ cells were more effective at detecting FMDV in clinical samples, supporting their use as a more sensitive tool for virus isolation.

Foot-and-mouth disease virus (FMDV) is a severe economic concern for meat-producing nations since the trade of animal products is limited in the countries where the virus is present. The rapid spread of the virus among susceptible animals results in severe morbidity and, in some cases, death, especially in young animals (reviewed in reference 1). Infection or vaccination with one of the seven different serotypes does not confer cross-protection to other serotypes or even to some subtypes of the same serotype. Vaccines for FMDV are widely used to prevent clinical disease, but since vaccines are serotype and subtype specific, the virus(es) causing outbreaks must be isolated and serologically characterized for vaccine matching prior to selecting the appropriate vaccine antigen to be used in vaccine formulations (reviewed in reference 2).

Although molecular techniques, such as PCR coupled with genomic sequencing, can be used in samples containing enough virus to rapidly identify the virus serotype and its relationship to other FMDV strains, appropriate vaccine prediction requires virus growth in cell culture to carry out neutralization tests using reference sera. Inefficient recovery of virus from animal samples can delay diagnosis and vaccine selection, hampering the rapid implementation of control measures; thus, virus isolation protocols are designed for maximum sensitivity. Some primary cells, such as bovine thyroid (BTY), are highly susceptible to a wide range of FMDV serotypes (3), but they are difficult and costly to prepare and lose FMDV susceptibility after multiple passages (4). Primary lamb kidney (LK) cells are also very sensitive to FMDV, and unlike BTY cells, LK cells maintain their sensitivity to FMDV infection after cryopreservation (5). Immortalized cell lines (e.g., baby hamster kidney [BHK-21] fibroblasts and porcine kidney epithelial cells), while much easier to maintain, are in many cases less susceptible to specific animal-derived FMDV serotypes (6–9). There is a need for a cell line that is easily maintained and is highly susceptible to all serotypes and subtypes of FMDV.

Integrins of the α_V subgroup have been demonstrated by several laboratories to be FMDV receptors, including our laboratory (reviewed in reference 10). Of the many α_V integrins that have been shown to mediate FMDV attachment, the integrin $\alpha_V\beta_6$ has been shown to be one of the most efficient receptors for all FMDV serotypes (11, 12), and the sites of infection in cattle show high levels of $\alpha_V\beta_6$ expression on epithelial cells (13, 14). BTY cells, considered the most sensitive primary cells for FMDV isolation, have high levels of $\alpha_V\beta_6$ integrin surface expression (15). Moreover, the transient expression of bovine α_v and β_6 integrin subunits in baby hamster kidney cells (BHK3-$\alpha_V\beta_6$) (16) greatly increased the susceptibility of this cell line to a cow-passaged FMDV A24 Cruzeiro strain that contains an SGD motif in the VP1 capsid protein (17). Although initially more permissive to the A24-SGD virus than BHK-21 cells, the BHK3-$\alpha_V\beta_6$ cells lost integrin expression and sensitivity to this virus after multiple passages (E. Rieder, personal communication).

Swaney (18) derived an immortalized line of fetal bovine kidney (LFBK) cells that had high susceptibility to most FMDV serotypes that was maintained over many passages. Compared to BTY cells, LFBK cells had similar susceptibilities to most FMDV serotypes and had equal or better susceptibility than MVPK, IB-RS-2, and fetal bovine kidney cells in the same experiments (18). In the work presented here, we combined the long-lived FMDV suscep-

Received 20 December 2012 **Returned for modification** 6 February 2013
Accepted 14 March 2013
Published ahead of print 20 March 2013
Address correspondence to Peter W. Krug, peter.krug@ars.usda.gov.
M.L. and P.W.K. contributed equally to this article.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.03370-12.

doi:10.1128/JCM.03370-12

tibility of the LFBK cell line with a principal bovine receptor for FMDV to derive a stable cell line that is highly susceptible to FMDV. We characterize the FMDV susceptibility of this transduced cell line by infection with animal-derived FMDV from all 7 serotypes, as well as from recent diagnostic field samples, and compared its susceptibility to those of other cell types used for diagnostic FMDV virus isolation. Our results indicate that LFBK-αvβ6 cells are highly permissive for all FMDV serotypes and have excellent performance in a diagnostic setting.

MATERIALS AND METHODS

Cells. LFBK cells (18) were propagated in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics as described previously (19) and were used for these experiments between passages 64 and 71. LFBK-$\alpha_V\beta_6$ cells were propagated in DMEM (catalog no. 11965092; Gibco) supplemented with 10% fetal bovine serum (catalog no. SH30071.03; HyClone) and antibiotics (catalog no. 15240062; Gibco) and were used at the passage indicated in each figure. BHK-21 cells, used between passages 62 and 67, were propagated in minimal essential medium (MEM) supplemented with 10% calf serum, 10% tryptose phosphate broth, nonessential amino acids, and antibiotics. Primary lamb kidney (LK) cells, generously supplied by the APHIS Diagnostic Service Section at the Plum Island Animal Disease Center (PIADC), were propagated in DMEM supplemented with 10% fetal bovine serum, antibiotics, and sodium pyruvate and were used directly from cryovials or at passage 1. IB-RS-2 cells were used between passages 129 and 137 and MVPK cells were used between passages 38 and 41; both of these cell lines were propagated in MEM supplemented with 10% fetal calf serum, nonessential amino acids, and antibiotics.

Transduction of LFBK cells with α_V and β_6 integrin subunits. Individual retroviruses expressing the bovine α_V (GenBank accession no. AF239958) or bovine β_6 (GenBank accession no. AF468060) integrin subunits (16) were created using the Pantropic retroviral expression system (PT3346-5; Clontech) as per the manufacturer's protocol. LFBK cells at passage 145 were infected with the α_V-expressing retrovirus, and neomycin selection was used to select against nontransduced cells. A pool of these cells was infected with the β_6-expressing retrovirus and was cloned to single cells by terminal dilution. A clone was chosen that showed consistent expression of the β_6 subunit by immunohistochemistry as described below.

Plaque assays. Cells were seeded 72 h before infection in 6-well cell culture plates unless otherwise noted. Ten-fold virus dilutions were made using phosphate-buffered saline containing calcium and magnesium supplemented with 1% bovine serum. The inoculum volume was 200 μl per well. One hour postadsorption, the inoculum was removed and the cells were overlaid with 50/50 1.2% gum tragacanth-2× MEM supplemented with antibiotic-antimycotic. Plates were incubated for 24 h unless otherwise indicated and simultaneously fixed and stained with HistoChoice tissue fixative (AMRESCO) containing crystal violet, and plaques were counted. Where indicated, statistical analysis was performed on log-transformed titer values using one-way analysis of variance (ANOVA) in Prism (GraphPad Software, Inc.).

Multistep growth curve. LFBK-$\alpha_V\beta_6$ or LFBK cells were seeded in 24-well plates 72 h before infection with either FMDV A24-BHK (a cell culture-passaged virus containing the wild-type RGD motif in VP1) or FMDV A24-SGD (vesicular fluid from the second bovine passage of the SGD-containing A24-B9 virus described in reference 17) at a multiplicity of 0.01 PFU/cell. Virus stocks were diluted in maintenance medium (DMEM supplemented with 1% fetal bovine serum and antibiotics) and absorbed for 1 h with rocking at 37°C. The cells were then acid washed (25 mM 2-(*N*-morpholino)ethanesulfonic acid [MES] [pH 5.5] in 145 mM NaCl), washed once with DMEM, and incubated at 37°C in 0.5 ml maintenance medium per well. At the indicated time, cells were frozen at −70°C and thawed, and the virus titer was determined by plaque assay on LFBK-$\alpha_V\beta_6$ cells.

Immunohistochemistry. The Vectastain ABC kit (PK-6102; Vector Laboratories) and Vector VIP peroxidase substrate kit (SK-4600; Vector Laboratories) were used according to the manufacturer's protocols. Mouse anti-human β_6 (MAB2076Z; Chemicon) was used to detect the bovine integrin subunit β_6 at a 1:300 dilution. The mouse monoclonal antibody F19-51 (20) was used to detect the FMDV 3D protein at a 1:200 dilution.

Detection of FMDV in diagnostic tissue samples. LK, BHK-21, IB-RS-2, and LFBK-$\alpha_V\beta_6$ cells were seeded onto 48-well cell culture plates 48 h prior to infection. LK cells were seeded directly from storage cryovials. LFBK-$\alpha_V\beta_6$ cells were seeded at passage 32, IB-RS-2 at passage 136, and BHK-21 at passage 66. Diagnostic lesion tissues were disrupted, and the virus was isolated after centrifugation through a Spin-X purification column (Costar) as described in reference 21. These tissue macerates were diluted 10-fold in DMEM supplemented with 25 mM HEPES and 0.5% fetal calf serum, and 100 μl was then used to infect each cell type for 1 h at 37°C. After adsorption, 200 μl of maintenance medium was added to each well and the plates were incubated at 37°C. Starting at 4 h postinfection (hpi), visual evidence of cytopathic effects was recorded every 2 h until 20 hpi and then at 24, 28, and 48 hpi. At 48 hpi, all wells were fixed with 50% acetone to 50% methanol and wells that were negative for cytopathic effects were immunostained with a monoclonal antibody to FMDV 3D protein to confirm the negative results.

RESULTS

Characterization of LFBK cells expressing bovine $\alpha_v\beta_6$ integrin. The integrin $\alpha_V\beta_6$ is an important receptor for FMDV in relevant tissues *in vivo*. LFBK cells are a transformed cell line that has high sensitivity to most FMDV serotypes but does not express high levels of β_6 integrin protein. In order to merge the enhanced susceptibility of $\alpha_V\beta_6$ expression with the transformed phenotype of LFBK cells, the bovine α_V and β_6 integrin subunits were transduced into LFBK cells as described in Materials and Methods. Immunohistochemical staining demonstrated long-term maintenance of enhanced β_6 expression in the LFBK-$\alpha_V\beta_6$ cells for ≥102 cell passages compared to the nontransduced LFBK cells (Fig. 1A to C).

FMDV A24-SGD, an FMDV A24 Cruzeiro strain serially passed in cattle, has an SGD motif in the G-H loop of VP1 and an enhanced infectivity in cells expressing the $\alpha_V\beta_6$ integrin (17). LFBK-$\alpha_V\beta_6$ cells at various subculture passages and nontransduced LFBK cells were infected with A24-SGD or a cell culture-grown control virus (A24-BHK) to confirm the long-term functional maintenance of the $\alpha_V\beta_6$ integrin. These experiments demonstrate that the LFBK-$\alpha_V\beta_6$ cells have a 2.5-log-increased susceptibility to A24-SGD compared to LFBK cells and that this increased susceptibility is maintained for >100 passages (Fig. 1D). The expression of surface proteins can be delayed due to trypsin treatment during cell subculture. To determine if increasing the time between seeding and infecting the cells had an effect on susceptibility, LFBK and LFBK-$\alpha_V\beta_6$ cells were seeded on plates at 24, 48, or 72 h prior to infection with the A24-SGD or A24-BHK virus. It was found that seeding the LFBK-$\alpha_V\beta_6$ cells 72 h prior to infection provided only slightly better sensitivity than seeding 24 h prior to infection (Fig. 1E). This result indicates that the LFBK-$\alpha_V\beta_6$ cells can be infected early after trypsin treatment with only a minimal loss in susceptibility. In order to show that the LFBK-$\alpha_V\beta_6$ cells support the normal growth progression of FMDV, a multistep growth curve analysis was performed (Fig. 1F). In this experiment, the replication of A24-BHK was similar in both LFBK and LFBK-$\alpha_V\beta_6$ cells. While A24-SGD replicated slowly in the nontransduced LFBK cells, this virus grew normally in LFBK-

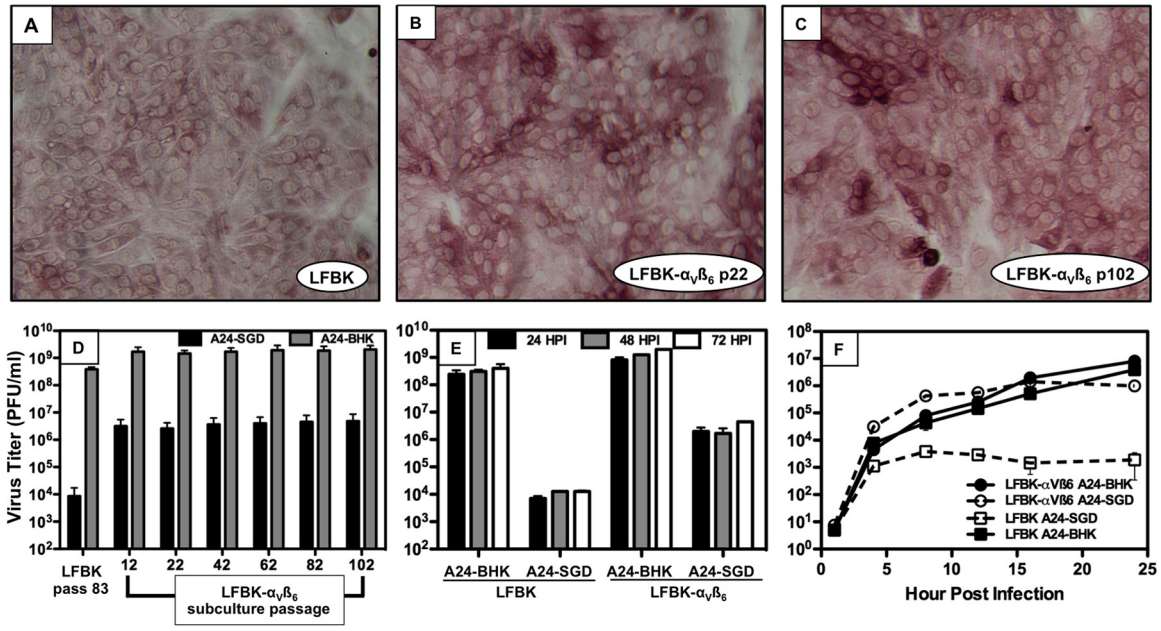

FIG 1 LFBK-$\alpha_V\beta_6$ cell characterization. LFBK-$\alpha_V\beta_6$ cells maintain long-term expression of β_6. Mouse anti-human β_6 was used to detect the bovine integrin subunit β_6 in LFBK at passage 83 (A), LFBK-$\alpha_V\beta_6$ at passage 22 (B), or passage 102 (C). Dark staining indicates β_6 expression. (D) LFBK-$\alpha_V\beta_6$ cells maintain long-term susceptibility to A24-SGD. The indicated cells were infected with serial dilutions of either A24-BHK or A24-SGD for plaque titration as described in Materials and Methods. (E) Relationship between susceptibility and time after seeding. Cells were seeded at the indicated time prior to infection and then infected with serial dilutions of the indicated virus for plaque titration as described in Materials and Methods. (F) Comparison of FMDV A24-BHK and A24-SGD growth in LFBK and LFBK-$\alpha_V\beta_6$ cells. LFBK and LFBK-$\alpha_V\beta_6$ cells were seeded in 24-well plates and infected 72 h later with either A24-BHK or A24-SGD at a multiplicity of infection (MOI) of 0.01. After 1 h, the monolayers were acid washed and fresh medium was added. At the indicated times after infection, the plates were frozen at −70°C, thawed, and titrated on LFBK-$\alpha_V\beta_6$ cells.

$\alpha_V\beta_6$ cells, demonstrating that the expression of $\alpha_V\beta_6$ in LFBK cells complements the defect of nontransduced LFBK cells to support efficient A24-SGD virus replication.

Susceptibility of LFBK-αVβ6 cells to animal-derived FMDV of all serotypes. In order to determine the susceptibility of LFBK-$\alpha_V\beta_6$ cells to animal-derived FMDV strains and compare it with those of LFBK, primary lamb kidney, BHK-21, and two swine kidney cells lines (IB-RS-2 and MVPK), each cell type was infected with serial dilutions of infected tissue macerates or vesicular fluid obtained from animals that were experimentally infected with each of the FMDV serotypes. The viruses used in this experiment were passaged twice in cattle or swine and are listed in Table 1. For each strain of virus, the LFBK-$\alpha_V\beta_6$ cells had equal or higher sensitivity to animal-derived FMDV than the other cells that we tested. In some cases, especially with the FMDV serotype O strains, the LFBK-$\alpha_V\beta_6$ cells supported FMDV replication that was >10-fold higher than most cell types tested (Fig. 2; see also Table S1 in the supplemental material). Overall, these data confirm that LFBK-$\alpha_V\beta_6$ cells can readily detect all FMDV serotypes in tissues from experimentally infected animals.

Detection of FMDV in diagnostic tissue samples using LFBK-$\alpha_V\beta_6$ cells. Samples from experimentally infected animals tend to have a high titer and better integrity than do field samples. To more closely mimic diagnostic conditions, we obtained field

TABLE 1 Experimentally infected animal-derived viruses used in this study

Virus strain	Sample species[a]	Sample type	Reference/source
A24 Cruzeiro/55	Swine	Vesicular fluid	Pacheco et al. (22)
A24 Cruzeiro/55	Bovine	Pool of bovine tongue and vesicular fluid	Uddowla et al. (23)
O1 Manisa/69	Bovine	Tongue tissue homogenate	Arzt et al. (24)
O1 Manisa/69	Swine	Vesicular fluid	Pacheco et al. (22)
Asia1 Shamir/89	Bovine	Tongue tissue homogenate	Science and Technology Directorate, DHS
Asia1 Shamir/89	Swine	Vesicular fluid	Pacheco et al. (22)
C3 Resende/55	Swine	Vesicular fluid	Nfon et al. (25)
C3 Resende/55	Bovine	Tongue tissue homogenate	Foreign Animal Disease Diagnostic Lab, APHIS
O/Taiwan/97	Swine	Vesicular fluid	Pacheco and Mason (29)
O/UK/35/01	Swine	Vesicular fluid	Pacheco and Mason (29)
SAT1/Zimbabwe/79	Bovine	Tongue tissue homogenate	Foreign Animal Disease Diagnostic Lab, APHIS
SAT2 Saudi Arabia/2000	Bovine	Vesicular fluid	Science and Technology Directorate, DHS
SAT3/Zimbabwe/83	Bovine	Tongue tissue homogenate	Science and Technology Directorate, DHS

[a] Each virus was collected from the indicated tissue(s) during the second round of infection in the indicated species.

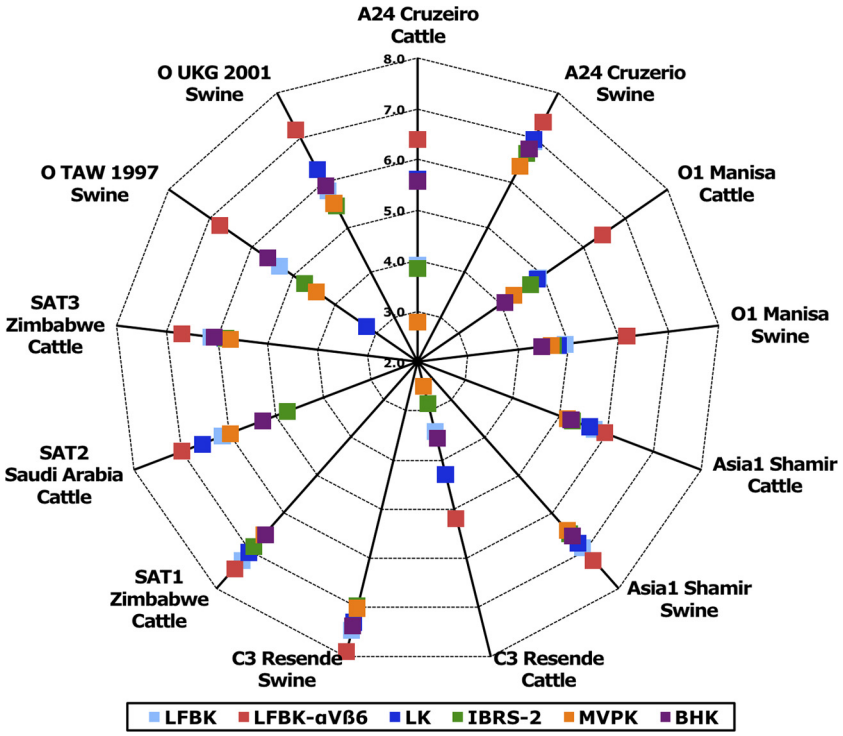

FIG 2 Susceptibility of 6 cell types to animal-derived FMDV. Cells were infected with the indicated viruses (Table 1), and virus titers were determined on each cell type by plaque assay. Each solid line is the axis for titers generated from the indicated virus strain. Colored points on the solid lines indicate the mean titer of that virus strain in a single cell type (see Table S1 in the supplemental material for sample range). Dashed lines specify the $\log_{10}$ scale of the virus titer in PFU/ml; points located outside the plot correspond to higher virus titers (10^8) than those located toward the center (10^2). Each point corresponds to the mean of ≥3 replicates.

diagnostic samples from Afghanistan, Bolivia, and Pakistan (see Table S2 in the supplemental material). These samples were processed from tissues according to standard virus isolation procedures and were used to infect LFBK-$\alpha_V\beta_6$ cells, as well as cells commonly used for FMDV diagnostics, including LK, IB-RS-2, and BHK-21 cells. Figure 3A shows the time point at which each diagnostic isolate first showed visible cytopathic effects (CPE) in each cell line. We observed that LFBK-$\alpha_V\beta_6$ was the only cell type in which all clinical samples were positive based on the presence of CPE by 24 h postinfection. A few of the samples yielded virus in all 4 cell lines, 12 samples were able to show CPE in only LFBK-$\alpha_V\beta_6$ or LK cells, and 3 of the 38 isolates tested grew only in LFBK-$\alpha_V\beta_6$ cells. These data show that LFBK-$\alpha_V\beta_6$ is the most permissive to these FMDV field isolates among all the cells tested. In order to rule out the presence of FMDV in noncytopathic infection, all CPE-negative wells were stained for FMDV 3D antigen after 48 hpi; none of the CPE-negative LK and IB-RS-2 wells reacted with the antibody. Five CPE-negative BHK-21 wells had individual nonrounded cells that stained positive for antigen (data not shown), indicating that these particular FMDV isolates were able to enter BHK-21 cells but were not spread efficiently in the culture by 48 h.

Figure 3B shows the overall CPE progression over time in all 38 virus isolates by plotting the mean CPE score of each isolate at each time point per cell type. On average, the LK and LFBK-$\alpha_V\beta_6$ cells performed very similarly, both being much more permissive to the diagnostic isolates than the IB-RS-2 and BHK-21 cells. While the LFBK-$\alpha_V\beta_6$ cells had slightly faster overall early detection of the isolates than the LK cells, the higher mean CPE score at later times indicates a faster progression of virus replication in the LFBK-$\alpha_V\beta_6$ cells. Taken together, the enhanced susceptibility, rapid initial detection of CPE, and faster progression of CPE strongly suggest that LFBK-$\alpha_V\beta_6$ cells are superior to many cells that are currently used for FMDV diagnostics from tissue specimens.

Susceptibility of LFBK-αVβ6 cells to other vesicular disease-causing viruses. Animals exhibiting vesicular lesions might be infected with other agents besides FMDV. In order to determine if LFBK-$\alpha_V\beta_6$ cells could detect other viruses causing vesicular disease, we inoculated BHK-21, LK, IB-RS-2, LFBK, or LFBK-$\alpha_V\beta_6$ cells with each of 5 non-FMD viruses that cause vesicular disease. We found that vesicular exanthema of swine virus (VESV) and swine vesicular disease virus (SVDV) replicated as well in LFBK-$\alpha_V\beta_6$ cells as they did in IB-RS-2 (Table 2). Vesicular stomatitis virus serotype New Jersey (VSV-NJ) grew to similar titers in all the cell lines. LK, LFBK, and LFBK-$\alpha_V\beta_6$ cells supported the growth of bovine papular stomatitis virus (BPSV), as evidenced by the formation of plaques; BPSV grew to a slightly higher titer in LK cells than in LFBK-$\alpha_V\beta_6$ cells. Infection with bluetongue virus induced cytopathic effects in only IB-RS-2 and LK cells by 96 hpi.

DISCUSSION

Here, we report the characterization of a bovine kidney cell line that is stably transduced with the bovine α_V and β_6 integrin subunits. The expression of the β_6 integrin subunit and the enhanced susceptibility to FMDV containing an SGD domain in VP1 were

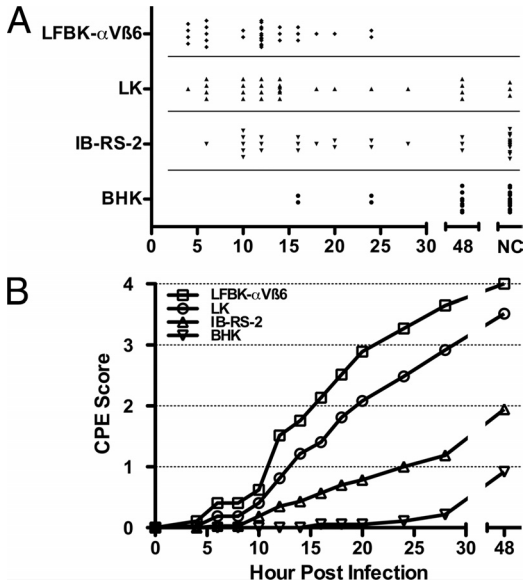

FIG 3 Detection of FMDV in diagnostic samples. Wells of 48-well plates seeded with the indicated cells were infected with 38 FMDV-suspect tissue homogenates from Afghanistan, Bolivia, and Pakistan (see Table S2 in the supplemental material for isolate information). Beginning at 4 h postinfection, each well was scored for the presence of cytopathic effects. (A) Each point on the scatter plot represents the time after infection that a cytopathic effect was first detected in a given well for one diagnostic isolate. NC denotes the diagnostic samples that did not induce visible cytopathic effects by 48 h in the indicated cells. (B) Each point on the graph represents the average CPE score for all 38 diagnostic isolates at each time point. 1+, ≤25% of the cell monolayer exhibited visual CPE; 2+, approximately 50% of the cell monolayer exhibited CPE; 3+, approximately 75% of the cell monolayer exhibited CPE; 4+, 100% of the cell monolayer exhibited CPE.

both maintained for ≥100 cell passages. We found that the LFBK-$\alpha_V\beta_6$ cells were more susceptible to all FMDV serotypes derived from experimentally infected animals than were other commonly used cells for FMDV isolation. In a diagnostic sensitivity assay, LFBK-$\alpha_V\beta_6$ cells were more sensitive than primary lamb kidney, IBRS-2, and BHK-21 cells. LFBK-$\alpha_V\beta_6$ cells were also able to detect other vesicular disease viruses. Our results support the use of LFBK-$\alpha_V\beta_6$ cells for FMDV diagnostic purposes.

Our data also indicate that the LFBK-$\alpha_V\beta_6$ cells are highly effective for the detection of FMDV serotype O isolates. A previous study (21) showed that LFBK cell sensitivity to FMDV O1 Manisa isolated from cattle was close to that of bovine tongue inoculation, and in our hands, the LFBK-$\alpha_V\beta_6$ cells detected O1 Manisa >1-$\log_{10}$-more efficiently than LFBK cells. Further, Burman and coworkers (26) showed that the integrin-binding domain on the VP1 capsid protein from O1 FMDV binds $\alpha_V\beta_6$ with the highest affinity among the $\alpha_V\beta_3$, $\alpha_V\beta_6$, and $\alpha_V\beta_8$ integrins. In our experiments (Fig. 2), O1 Manisa viruses were detected at titers nearly 2 logs better in LFBK-$\alpha_V\beta_6$ than in IB-RS-2 cells that do not express high levels of $\alpha_V\beta_6$ (15).

BHK-21 cells are widely used for FMDV propagation in research laboratory settings due to their rapid growth properties and sufficient susceptibility to most serotypes of FMDV. BHK-21 cells generally performed well in our sensitivity experiments (Fig. 2), detecting cattle-derived A24, as well as primary LK cells; O1 Manisa (from both swine and cattle) was the only serotype where BHK-21 had the least efficient FMDV detection of the cell lines tested. In contrast, low-passage-number BHK cells were able to detect FMDV from only 18 out of 40 diagnostic tissue samples, and in five of those cases, the detection of a few single infected BHK-21 cells (most with no obvious cytopathic morphology) was done by immunohistochemistry. Taken together, our results clearly indicate that BHK-21 cells are not as susceptible to animal-derived FMDV isolates as LFBK-$\alpha_V\beta_6$ cells.

FMDV strain O/TAW/97 does not grow well in primary bovine thyroid cells (BTY) or in cattle, and historically, its detection requires the use of swine cells, such as IB-RS-2 (27). As such, O/TAW/97 has been referred to as a porcinophilic virus (27–29). In our experiments, this virus grew poorly in primary LK cells, forming extremely small plaques. Interestingly, in our hands, O/TAW/97 grew better in the bovine-derived LFBK cells than in the swine-derived MVPK and IB-RS-2 cell lines. This suggests that the growth defect of O/TAW/97 in BTY (and possibly LK) cells is a postattachment step, since BTY cells express the $\alpha_V\beta_6$ integrin, indicating that other cell-specific factors are involved. Indeed, alterations in the 3A protein of O/TAW/97 have been identified (28), and while the function of 3A is not clear (30–32), it seems to be involved in host range specificity (33, 34). The reason that LFBK and LFBK-$\alpha_V\beta_6$ support the replication of this virus is unknown, but we can speculate that these cells have lost a factor that is present in bovine cells that restricts O/TAW/97 growth. Although we have not made a direct comparison, the results presented here suggest that LFBK-$\alpha_V\beta_6$ cells can support the replication of O/TAW/97 more efficiently than BTY cells.

IB-RS-2 cells have been shown to be less sensitive to some serotypes of FMDV than primary bovine thyroid cells (7), possibly due to low levels of $\alpha_V\beta_6$ integrin on the surface of the cells (15). In our experiments, the IB-RS-2 and MVPK cells had poor sensitivities to certain serotypes from bovine tissues, especially A24 Cruzeiro, O1 Manisa, and C3 Resende, whereas the A24 and C3 viruses from porcine tissues were efficiently detected by both swine origin cell lines. Interestingly, IB-RS-2, but not MVPK, cells were specifically less sensitive to SAT2 virus isolated from cattle; unfortunately, a comparison to swine-derived SAT2 was not available. While many laboratories include IB-RS-2 cells for the detection of swine vesicular diseases and swine-adapted FMDV strains, one concern with the diagnostic use of these cells is that they can test positive for classical swine fever (35), potentially confounding the results in array-based diagnostic assays.

Recently, Brehm and coworkers demonstrated the sensitivity of a fetal goat tongue cell line (36) that supports the replication of all FMDV strains tested, with the exception of the porcinophilic

TABLE 2 Growth of selected animal-derived vesicular disease viruses in various cell types

Virus (strain)	Titer[a] of the indicated virus in the following cell types:				
	LFBK	LFBK-$\alpha_V\beta_6$	IB-RS-2	BHK-21	LK
VESV (A48ET305)	6.15[a]	6.55	5.8	ND[b]	3.05
SVDV (UKG72)	6.2	6.2	6.3	ND	3.05
BSPV (New York 2004)	3.2	3.05	3.2	ND	3.8
BTV-1 (S. Africa 1993)	ND	ND	3.2	ND	3.05
VSV (New Jersey[c])	7.3	7.05	6.55	6.3	5.8

[a] Virus titers in $\log_{10}$ 50% tissue culture infective dose ($TCID_{50}$)/ml.
[b] ND, not detected. Limit of detection in this assay is 1.8 $\log_{10}$ $TCID_{50}$/ml.
[c] This virus was obtained from a pool of experimentally infected animal tissues.

O/TAW/97 strain. According to the authors, this cell line expresses the $\alpha_V\beta_6$ integrin and is equal to primary BTY cells in its sensitivity to FMDV. Although these cells were reported to grow slowly in culture, the monolayers of these goat tongue cells take days to become confluent but then are stable for a period of weeks once established. In contrast, the LFBK-$\alpha_V\beta_6$ cells grow very quickly in culture and are readily susceptible to infection within 24 h after seeding.

LFBK-$\alpha_V\beta_6$ cells have been used for FMDV research for several years at PIADC. They support the replication of animal-derived virus strains that do not grow well in other cell types (e.g., O1 Manisa and O/TAW/97) and maintain a high sensitivity to FMDV for >100 subculture passages. They do not require the extraction of animal organs to make primary cells, they grow as efficiently as standard LFBK cells, and they have no special medium requirements. Based on the data presented here, LFBK-$\alpha_V\beta_6$ is an excellent cell line for FMDV diagnostic- and research-based cell applications. LFBK-$\alpha_V\beta_6$ cells have been deposited in the American Type Culture Collection (PTA-13047).

ACKNOWLEDGMENTS

We thank the following groups for their assistance obtaining the viruses and for other reagents used in this study: the APHIS Foreign Animal Disease Diagnostics Laboratory at PIADC for primary lamb kidney cells, field isolates of FMDV, and field isolates of VESV, SVDV, bluetongue virus 1 (BTV-1), and BPSV, the Department of Homeland Security Science and Technology Directorate for experimentally infected animal-derived isolates of FMDV, and Khalid Naeem of the National Agricultural Research Center in Islamabad, Pakistan, for field isolates of FMDV. We thank Steve Pauszek (ARS) for serotype determination of the field isolates from Pakistan.

The LFBK-$\alpha_V\beta_6$ cell line is the subject of a U.S. patent application.

REFERENCES

1. **Grubman MJ, Baxt B.** 2004. Foot-and-mouth disease. Clin. Microbiol. Rev. **17**:465–493.
2. **Rodriguez LL, Gay CG.** 2011. Development of vaccines toward the global control and eradication of foot-and-mouth disease. Expert Rev. Vaccines **10**:377–387.
3. **Snowdon WA.** 1966. Growth of foot-and-mouth disease virus in monolayer cultures of calf thyroid cells. Nature **210**:1079–1080.
4. **House JA, Yedloutschnig RJ.** 1982. Sensitivity of seven different types of cell cultures to three serotypes of foot-and-mouth disease virus. Can. J. Comp. Med. **46**:186–189.
5. **House C, House JA.** 1989. Evaluation of techniques to demonstrate foot-and-mouth disease virus in bovine tongue epithelium: comparison of the sensitivity of cattle, mice, primary cell cultures, cryopreserved cell cultures and established cell lines. Vet. Microbiol. **20**:99–109.
6. **Swaney LM.** 1976. Susceptibility of a new fetal pig kidney cell line (MVPK-1) to foot-and-mouth disease virus. Am. J. Vet. Res. **37**:1319–1322.
7. **Ferris NP, King DP, Reid SM, Hutchings GH, Shaw AE, Paton DJ, Goris N, Haas B, Hoffmann B, Brocchi E, Bugnetti M, Dekker A, De Clercq K.** 2006. Foot-and-mouth disease virus: a first inter-laboratory comparison trial to evaluate virus isolation and RT-PCR detection methods. Vet. Microbiol. **117**:130–140.
8. **Ferris NP, Hutchings GH, Moulsdale HJ, Golding J, Clarke JB.** 2002. Sensitivity of primary cells immortalised by oncogene transfection for the detection and isolation of foot-and-mouth disease and swine vesicular disease viruses. Vet. Microbiol. **84**:307–316.
9. **De Castro MP.** 1964. Behaviour of the foot-and-mouth disease virus in cell cultures: susceptibility of the IB-RS-2 line. Arch. Inst. Biol., São Paulo. **31**:63–78.
10. **Ruiz-Sáenz J, Goez Y, Tabares W, López-Herrera A.** 2009. Cellular receptors for foot and mouth disease virus. Intervirology **52**:201–212.
11. **Jackson T, Sheppard D, Denyer M, Blakemore W, King AMQ.** 2000. The epithelial integrin $\alpha_V\beta_6$ is a receptor for foot-and-mouth disease virus. J. Virol. **74**:4949–4956.
12. **Ferris NP, Abrescia NGA, Stuart DI, Jackson T, Burman A, King DP, Paton DJ.** 2005. Utility of recombinant integrin $\alpha_V\beta_6$ as a capture reagent in immunoassays for the diagnosis of foot-and-mouth disease. J. Virol. Methods **127**:69–79.
13. **Monaghan P, Gold S, Simpson J, Zhang Z, Weinreb PH, Violette SM, Alexandersen S, Jackson T.** 2005. The alpha(v)beta6 integrin receptor for foot-and-mouth disease virus is expressed constitutively on the epithelial cells targeted in cattle. J. Gen. Virol. **86**:2769–2780.
14. **O'Donnell V, Pacheco JM, Gregg D, Baxt B.** 2009. Analysis of foot-and-mouth disease virus integrin receptor expression in tissues from naïve and infected cattle. J. Comp. Pathol. **141**:98–112.
15. **King DP, Burman A, Gold S, Shaw AE, Jackson T, Ferris NP.** 2011. Integrin sub-unit expression in cell cultures used for the diagnosis of foot-and-mouth disease. Vet. Immunol. Immunopathol. **140**:259–265.
16. **Duque H, LaRocco M, Golde WT, Baxt B.** 2004. Interactions of foot-and-mouth disease virus with soluble bovine alphaVbeta3 and alphaV-beta6 integrins. J. Virol. **78**:9773–9781.
17. **Rieder E, Henry T, Duque H, Baxt B.** 2005. Analysis of a foot-and-mouth disease virus type A24 isolate containing an SGD receptor recognition site *in vitro* and its pathogenesis in cattle. J. Virol. **79**:12989–12998.
18. **Swaney LM.** 1988. A continuous bovine kidney cell line for routine assays of foot-and-mouth disease virus. Vet. Microbiol. **18**:1–14.
19. **Piccone ME, Feng Y, Chang AC, Mosseri R, Lu Q, Kutish GF, Lu Z, Burrage TG, Gooch C, Rock DL, Cohen SN.** 2009. Identification of cellular genes affecting the infectivity of foot-and-mouth disease virus. J. Virol. **83**:6681–6688.
20. **Yang M, Clavijo A, Li M, Hole K, Holland H, Wang H, Deng MY.** 2007. Identification of a major antibody binding epitope in the non-structural protein 3D of foot-and-mouth disease virus in cattle and the development of a monoclonal antibody with diagnostic applications. J. Immunol. Methods **321**:174–181.
21. **Pacheco JM, Arzt J, Rodriguez LL.** 2010. Early events in the pathogenesis of foot-and-mouth disease in cattle after controlled aerosol exposure. Vet. J. **183**:46–53.
22. **Pacheco JM, Tucker M, Hartwig E, Bishop E, Arzt J, Rodriguez LL.** 2012. Direct contact transmission of three different foot-and-mouth disease virus strains in swine demonstrates important strain-specific differences. Vet. J. **193**:456–463.
23. **Uddowla S, Hollister J, Pacheco JM, Rodriguez LL, Rieder E.** 2012. A safe foot-and-mouth disease vaccine platform with two negative markers for differentiating infected from vaccinated animals. J. Virol. **86**:11675–11685.
24. **Arzt J, Pacheco JM, Rodriguez LL.** 2010. The early pathogenesis of foot-and-mouth disease in cattle after aerosol inoculation. Identification of the nasopharynx as the primary site of infection. Vet. Pathol. **47**:1048–1063.
25. **Nfon CK, Toka FN, Kenney M, Pacheco JM, Golde WT.** 2010. Loss of plasmacytoid dendritic cell function coincides with lymphopenia and viremia during foot-and-mouth disease virus infection. Viral Immunol. **23**:29–41.
26. **Burman A, Clark S, Abrescia NG, Fry EE, Stuart DI, Jackson T.** 2006. Specificity of the VP1 GH loop of foot-and-mouth disease virus for alphav integrins. J. Virol. **80**:9798–9810.
27. **Dunn CS, Donaldson AI.** 1997. Natural adaption to pigs of a Taiwanese isolate of foot-and-mouth disease virus. Vet. Rec. **141**:174–175.
28. **Knowles NJ, Davies PR, Henry T, O'Donnell V, Pacheco JM, Mason PW.** 2001. Emergence in Asia of foot-and-mouth disease viruses with altered host range: characterization of alterations in the 3A protein. J. Virol. **75**:1551–1556.
29. **Pacheco JM, Mason PW.** 2010. Evaluation of infectivity and transmission of different Asian foot-and-mouth disease viruses in swine. J. Vet. Sci. **11**:133–142.
30. **Moffat K, Howell G, Knox C, Belsham GJ, Monaghan P, Ryan MD, Wileman T.** 2005. Effects of foot-and-mouth disease virus nonstructural proteins on the structure and function of the early secretory pathway: 2BC but not 3A blocks endoplasmic reticulum-to-Golgi transport. J. Virol. **79**:4382–4395.
31. **García-Briones M, Rosas MF, González-Magaldi M, Martín-Acebes MA, Sobrino F, Armas-Portela R.** 2006. Differential distribution of non-structural proteins of foot-and-mouth disease virus in BHK-21 cells. Virology **349**:409–421.
32. **O'Donnell VK, Pacheco JM, Henry TM, Mason PW.** 2001. Subcellular

distribution of the foot-and-mouth disease virus 3A protein in cells infected with viruses encoding wild-type and bovine-attenuated forms of 3A. Virology **287**:151–162.
33. **Rosas MF, Vieira YA, Postigo R, Martín-Acebes MA, Armas-Portela R, Martínez-Salas E, Sobrino F.** 2008. Susceptibility to viral infection is enhanced by stable expression of 3A or 3AB proteins from foot-and-mouth disease virus. Virology **380**:34–45.
34. **Pacheco JM, Henry TM, O'Donnell VK, Gregory JB, Mason PW.** 2003. Role of nonstructural proteins 3A and 3B in host range and pathogenicity of foot-and-mouth disease virus. J. Virol. **77**:13017–13027.
35. **Bolin SR, Black JW, Frey ML, Katz JB, Ridpath JF, Roblin RO.** 1994. Detection of a cell line contaminated with hog cholera virus. J. Am. Vet. Med. Assoc. **205**:742–745.
36. **Brehm KE, Ferris NP, Lenk M, Riebe R, Haas B.** 2009. Highly sensitive fetal goat tongue cell line for detection and isolation of foot-and-mouth disease virus. J. Clin. Microbiol. **47**:3156–3160.

JCM
Journals.ASM.org

AUTHOR CORRECTION

Correction for LaRocco et al., A Continuous Bovine Kidney Cell Line Constitutively Expressing Bovine $\alpha_V\beta_6$ Integrin Has Increased Susceptibility to Foot-and-Mouth Disease Virus

Michael LaRocco,[a] Peter W. Krug,[a] Ed Kramer,[b] Zaheer Ahmed,[a] Juan M. Pacheco,[a] Hernando Duque,[c] Barry Baxt,[a] Luis L. Rodriguez[a]

Foreign Animal Disease Research Unit, United States Department of Agriculture, Agricultural Research Service, Plum Island Animal Disease Center, Greenport, New York, USA[a]; The McConnell Group, Inc., Rockville, Maryland, USA[b]; Foreign Animal Disease Diagnostic Laboratory, United States Department of Agriculture, Animal and Plant Health Inspection Service, Plum Island Animal Disease Center, Greenport, New York, USA[c]

Volume 51, no. 12, p. 1714–1720, 2012. After the initial distribution of the LFBK-$\alpha_V\beta_6$ cell line, studies were conducted to further characterize the cell line. These studies revealed that the LFBK parental cell line and the transduced LFBK-$\alpha_V\beta_6$ cell line are of porcine genotype and not of bovine origin as stated in the published article. This was confirmed by isoenzyme and real-time reverse transcription-PCR studies. The results presented in the published article describing the cell line remain unchanged except for the statements indicating that the cells are of bovine origin.

Citation LaRocco M, Krug PW, Kramer E, Ahmed Z, Pacheco JM, Duque H, Baxt B, Rodriguez LL. 2015. Correction for LaRocco et al., A continuous bovine kidney cell line constitutively expressing bovine $\alpha_V\beta_6$ integrin has increased susceptibility to foot-and-mouth disease virus. J Clin Microbiol 53:755. doi:10.1128/JCM.03220-14.

doi:10.1128/JCM.03220-14