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African Green Monkey Kidney (Vero) Cells Provide an Alternative Host Cell System for Influenza A and B Viruses

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The preparation of live, attenuated human influenza virus vaccines and of large quantities of inactivated vaccines after the emergence or reemergence of a pandemic influenza virus will require an alternative host cell system, because embryonated chicken eggs will likely be insufficient and suboptimal. Preliminary studies indicated that an African green monkey kidney cell line (Vero) is a suitable system for the primary isolation and cultivation of influenza A viruses (E. A. Govorkova, N. V. Kaverin, L. V. Gubareva, B. Meignier, and R. G. Webster, J. Infect. Dis. 172:250–253, 1995). We now demonstrate for the first time that Vero cells are suitable for isolation and productive replication of influenza B viruses and determine the biological and genetic properties of both influenza A and B viruses in Vero cells; additionally, we characterize the receptors on Vero cells compared with those on Madin-Darby canine kidney (MDCK) cells. Sequence analysis indicated that the hemagglutinin of Vero cell-derived influenza B viruses was identical to that of MDCK-grown counterparts but differed from that of egg-grown viruses at amino acid positions 196 to 198. Fluorescence-activated cell sorting analysis showed that although Vero cells possess predominantly α2,3 galactose-linked sialic acid, they are fully susceptible to infection with either human influenza A or B viruses. Moreover, all virus-specific polypeptides were synthesized in the same proportions in Vero cells as in MDCK cells. Electron microscopic and immunofluorescence studies confirmed that infected Vero cells undergo the same morphological changes as do other polarized epithelial cells. Taken together, these results indicate that Vero cell lines could serve as an alternative host system for the cultivation of influenza A and B viruses, providing adequate quantities of either virus to meet the vaccine requirements imposed by an emerging pandemic.

Although used routinely to prepare human influenza virus vaccines and diagnostic reagents, embryonated chicken eggs have potentially serious limitations as a host system, including the lack of reliable year-round supplies of high-quality eggs and the low susceptibility of summer eggs to influenza virus infection (17). Moreover, the cultivation of influenza A and B viruses in eggs can lead to the selection of variants characterized by antigenic and structural changes in the hemagglutinin (HA) molecule (9, 23, 27), and the HA of clinical virus is essentially homogeneous and identical to that of the virus grown in mammalian cells (10, 22). Finally, the presence of adventitious agents in eggs can jeopardize the preparation of live, attenuated influenza virus vaccines.

Influenza viruses are able to replicate in a variety of primary, diploid, and continuous cell cultures (13), although the susceptibility of most cell lines to influenza virus infection is low. Human influenza viruses preferentially attach to sialic acid (SA) with α2,6 galactose (α2,6 Gal) oligosaccharides (1, 24); however, the distribution of these receptors on most mammalian cells has not been determined, and their influence on virus attachment and replication is unclear. Among the mammalian cell lines tested for cultivation of influenza A and B viruses, only MDCK cells were found to support optimal growth and isolation of virus (3, 26), but this line has not been licensed as a substrate for vaccine production. One of the candidates, the well-characterized African green monkey kidney (Vero) cell line, is suitable for the production of a number of human virus vaccines, including those against poliomyelitis and rabies (16). Earlier studies indicated that influenza viruses do not replicate productively in Vero cells (2, 15, 18, 29). The repeated addition of trypsin to the culture medium permits multicycle replication and the generation of high virus yields (12), and preliminary studies with a limited number of strains indicate that Vero cells support the primary isolation and replication of influenza A viruses (4).

In the present study, we characterized the influenza virus receptors on Vero cells, including their distribution, and showed that neither amino acid sequences of the HA and protein synthesis nor the morphology of viruses grown in Vero cells differs appreciably from findings in MDCK cells. We also establish that Vero cells are suitable for cultivation of influenza B viruses.

MATERIALS AND METHODS

Cells. The African green monkey kidney cell line (Vero) was obtained from the American Type Culture Collection, Rockville, Md. Vero and MDCK cells were cultivated as described previously (4). Plaque assays were performed with L-*l*-tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin (2.5 μg/ml; Worthington Diagnostics Freehold, N.J.) as described previously (6).

Virus replication. The replicative properties of egg-grown influenza A and B viruses, obtained from the repository at St. Jude Children's Research Hospital, were determined in Vero cells; the maintenance medium contained 1.0 μg of TPCK-treated trypsin per ml added at 0, 24, and 48 h postinfection. The virus yield was determined by HA and PFU titer determination in culture medium after incubation for 72 or 96 h at 33°C (for influenza B viruses) or 37°C (for influenza A viruses). The 50% tissue culture infectious doses $(TCID)_{50}$ per milliliter and 50% egg infectious doses per milliliter were calculated as described by Kärber (8).

Primary isolation. Vero and MDCK cells were infected with the throat washings of patients with clinical signs of influenza. Only samples that were positive for influenza virus by indirect immunofluorescence test or by previous isolation in eggs or MDCK cells were used.

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Adaptation of influenza viruses to Vero cells. Influenza viruses were adapted to Vero cells as previously described (4). A/England/1/53 (H1N1) [high growth] and B/Ann Arbor/1/86 strains were passaged 20 times at limiting dilutions.

Antigenic analysis. Monoclonal antibodies to the HA of egg-grown A/Baylor/11515/82 (H1N1), A/Baylor/5700/82 (H1N1), B/Ann Arbor/1/86, B/Memphis/6/86, and MDCK-grown B/Memphis/6/86 viruses were used in hemagglutination inhibition tests for antigenic characterization of the influenza A and B viruses.

Analysis of the abundance of SA α2,3 Gal and SA α2,6 Gal linkages on Vero and MDCK cells. The abundance of α2,3 Gal and α2,6 Gal linkages on Vero and MDCK cells was determined with the digoxigenin glycan differentiation kit (Boehringer Mannheim Biochemica, Mannheim, Germany). Briefly, Vero or MDCK cells were washed twice in phosphate-buffered saline (PBS) containing 10 mM glycine and then once with buffer 1 (50 mM Tris-HCl, 0.15 M NaCl, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM $CaCl_2$ [pH 7.5]). Digoxigenin-labeled lectins, *Sambus nigra* agglutinin (SNA) (specific for SA α2,6 Gal), and *Maackia amurensis* agglutinin (MAA) (specific for SA α2,3 Gal) were dissolved in buffer 1 and then incubated with the cells for 1 h at room temperature. After three washes, the cells were incubated with anti-digoxigenin-fluoresceinated Fab fragments (1:40 diluted in buffer 1) for 1 h at room temperature. After three washes, the cells were analyzed for relative fluorescence intensity on a FACScan fluorospectrometer (Becton Dickinson).

Surface immunofluorescence. Vero or MDCK cells were infected with A/England/1/53 (H1N1) or B/Ann Arbor/1/86 at 0.1, 1.0, or 10.0 PFU per cell. At 6 h postinfection, the cell suspensions were removed and fixed with 4% paraformaldehyde–PBS at room temperature. The cells were then processed for immunofluorescence (21) and were analyzed on a FACScan fluorospectrometer.

Protein gel electrophoresis and radioimmunoprecipitation. Vero or MDCK cells were infected with either A/England/1/53 (H1N1) or B/Ann Arbor/1/86 at a multiplicity of infection (MOI) of ~30 PFU per cell. After adsorption for 1 h at 37°C in virus growth medium, the cells were washed and incubated for 5 h (influenza A strain) or 8 h (influenza B strain) in the labeling medium (Dulbecco's modified Eagle's medium without methionine or cystine [ICN Biomedicals, Inc., Costa Mesa, Calif.]). Viral proteins were radioactively labeled with Tran[^{35}S]methionine/cysteine (100 μCi/ml; ICN Chemicals) for 2 h at 37°C. Viral proteins—HA, nucleoprotein (NP), nonstructural (NS1), and matrix (M1 and M2)—were analyzed by radioimmunoprecipitation with specific monoclonal antibodies as previously described (14). Monoclonal antibodies to the M2 protein (14 C2) were generously provided by R. A. Lamb, Northwestern University, Evanston, Ill. Imaging and quantitation of sodium dodecyl sulfate polyacrylamide gel electrophoresis (PAGE) gels (15% polyacrylamide) were performed with a Molecular Dynamics phosphorimager and Image Quant Software, respectively.

Gene amplification. RNA extraction, cDNA preparation, and PCR were performed as previously described (5).

Nucleotide sequence determination. Nucleotide sequencing was performed by the dideoxynucleotide chain termination method (25) with the fmol DNA sequencing system (Promega) and end-labeled primers.

Electron microscopy. Vero and MDCK cell monolayers were infected with Vero-adapted influenza strains [A/England/1/53 (H1N1) and B/Ann Arbor/1/86] at a MOI of 0.001 PFU per cell. Infected and control cell monolayers were fixed at 48 h postinfection in cacodylate-buffered 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in graded series of alcohols, and embedded in Spurr low-viscosity embedding medium (Ladd Research Industries, Burlington, Vt.). Ultrathin sections of cells were cut with a diamond knife on a Sorvall MT 6000 ultramicrotome, and the sections were examined in a Philips EM 301 electron microscope operated at 80 kV.

Immunohistochemical assay. To detect apoptotic changes in virus-infected Vero and MDCK cells, we used a commercial kit (Apoptag; ONCOR, Gaithersburg, Md.) as specified by the manufacturer.

RESULTS

Primary isolation and replication of influenza A and B viruses in Vero cells. High rates of primary isolation of influenza A virus from clinical specimens ($n = 27$) were obtained with Vero and MDCK cells but not with eggs: 70.4, 74.1, and 14.8%, respectively (Table 1). By contrast, the percentage of specimens ($n = 21$) yielding influenza B virus was comparable in Vero cells (47.6%) and eggs (42.9%) but higher in MDCK cells (57.1%). Ranges of $TCID_{50}$ titers were essentially the same whether viruses were grown in Vero or MDCK cells or eggs (Table 1).

The replicative capacity of 72 egg-grown influenza A and 11 influenza B virus strains was monitored over two serial passages in Vero cells. More than 90.0% of human, lower animal, and avian influenza A virus strains produced a detectable yield after the first passage by HA testing, compared with 51.4% after the second passage. Of the 11 influenza B virus strains tested, 10 were able to replicate in Vero cells after the first passage and 7 were able to do so after the second passage. Log_{10} $TCID_{50}$ per milliliter ranged from 1.7 to 5.2 for influenza A viruses and from 1.7 to 4.2 for influenza B viruses after the second passage.

The influenza A/England/1/53 (H1N1) and B/Ann Arbor/1/86 virus strains, which showed the best growth in Vero cells, were chosen for further studies designed to enhance virus yield by serial passaging at a low MOI (0.01 to 0.001 PFU per cell) in the presence of trypsin; it was shown previously (4) that after 20 passages of A/England/1/53 (H1N1) in Vero cells, the titer of infectious virus was 8.4 log_{10} $TCID_{50}$/ml. By the 20th passage in Vero cells, we noted an increase in the initial infectivity from 4.7 to 7.5 log_{10} $TCID_{50}$/ml for the B/Ann Arbor/1/86 strain, with $TCID_{50}$ titers that exceeded those in MDCK cells and were as high as those in eggs (results not shown).

Antigenic and genetic stability of the HA1 of influenza virus strains isolated and grown in different host cell systems. To determine whether replication of influenza A and B viruses in the Vero cell line selects host cell-mediated variants, we performed antigenic and sequence analyses of the HA1 molecule. Hemagglutination inhibition testing with polyclonal and anti-HA monoclonal antibodies revealed that the egg-grown B/Ann Arbor/1/86 strain was antigenically stable in Vero cells, because it retained its original antigenic characteristics during 20 passages in the mammalian host cell system (results not shown).

It was also important to determine if growth in Vero cells selected variant virus populations during primary isolation. We therefore compared the HA1 sequence of influenza B viruses (B/Memphis/1/93) isolated in Vero or MDCK cells or eggs. The virus isolated and grown in eggs has an amino acid sub-

TABLE 1. Primary isolation of influenza A and B viruses from clinical samples

Cell system	Clinical samples yielding[a]:			
	Influenza A virus ($n = 27$)		Influenza B virus ($n = 21$)	
	No. (%) HA positive	Infectivity range ($log_{10}TCID_{50}$/ml)	No. (%) HA positive	Infectivity range ($log_{10}TCID_{50}$/ml)
Vero	19 (70.4)	2.2–2.7	10 (47.6)	2.0–2.2
MDCK	20 (74.1)	2.8–3.1	12 (57.1)	2.7–3.0
Eggs	4 (14.8)	2.6–3.0	9 (42.9)	2.0–2.2

[a] HA positivity and $TCID_{50}$ titers were determined at 72 to 96 h postinfection after the second passage in the cell system tested. $TCID_{50}$ titers were determined in MDCK cells.

TABLE 2. Efficiency of influenza A or B virus infection of Vero and MDCK cells

Cell system	% of cells expressing HA at 6 h postinfection at different MOIs[a]					
	A/England/1/53 (H1N1)			B/Ann Arbor/1/86		
	0.1 PFU/cell	1.0 PFU/cell	10.0 PFU/cell	0.1 PFU/cell	1.0 PFU/cell	10.0 PFU/cell
Vero	2.9	45.2	87.1	1.7	16.7	81.2
MDCK	8.7	45.4	91.0	2.6	25.7	85.1

[a] The percentage of cells expressing HA was calculated according to the relative fluorescence intensity on a FACScan fluorospectrometer.

stitution with respect to findings in Vero- and MDCK-grown viruses. The egg-grown virus had Asn-Lys-Ala amino acid sequences at positions 196 to 198, while the mammalian cell-isolated counterparts maintained a potential carbohydrate site chain at the head of the HA molecule with a sequence of Asn-Lys-Thr. The HA1 region of influenza B/Memphis/1/93 virus isolated and passaged in Vero cells was indistinguishable from that in the MDCK-grown counterpart.

Receptor specificity of Vero cells and efficiency of influenza virus infection. To characterize the nature and relative abundance of influenza virus-binding receptors on the surface of Vero cells, we used fluorescence-activated cell sorter (FACS) analysis with two linkage-specific lectins for either *N*-acetylneuraminic acid α2,3 Gal (NeuAc α2,3 Gal) or NeuAc α2,6 Gal.

MAA which is specific for NeuAc α2,3 Gal, bound strongly to Vero cells (~93.0% of cells), whereas SNA, which recognizes NeuAc α2,6 Gal, bound to the surface of approximately 21.0% of the Vero cells. Thus, Vero cells are heterogeneous with respect to receptor binding. On the other hand, MDCK cells were stained with both types of linkage-specific lectins. Thus, Vero cells probably comprise two positive populations of receptor-bearing cells, one (~21.0% of cells) containing both types of sialyloligosaccharides specific for influenza viruses and the other containing primarily NeuAc α2,3 Gal.

Conceivably, the relative abundance of NeuAc α2,6 Gal linkages on Vero cells could affect the efficiency of infection by influenza A and B viruses. We tested this prediction by determining the percentage of cells expressing the HA molecule at different MOIs at 6 h postinfection (Table 2). The results of FACS analysis showed similar efficiencies of infection for Vero and MDCK cells infected with either influenza A or B viruses. At an MOI of 10.0 PFU per cell, 87.1% of Vero and 91.0% of MDCK cells were infected with the influenza A/England/1/53 virus strain. Slightly smaller percentages of cells expressing the HA were infected by the influenza B/Ann Arbor/1/86 virus: 81.2% for Vero and 85.1% for MDCK (Table 2). Similar correlations were observed between Vero and MDCK cells at MOIs of 0.1 and 1.0 PFU per cell.

Protein synthesis in Vero and MDCK cells infected with influenza A and B viruses. We also thought it important to assess the pattern of protein synthesis in Vero versus MDCK cells. To do so, we analyzed the protein synthesis in Vero cells infected with either influenza A/England/1/53 or B/Ann Arbor/1/86 virus compared with that in infected MDCK cells. The protein patterns of A/England/1/53-infected Vero cells demonstrated that most virus-specific polypeptides are synthesized in proportions similar to those in MDCK cells (Fig. 1A). There were no differences in electrophoretic migration of viral proteins synthesized in the two cell lines infected with the parental or Vero cell-adapted influenza A/England/1/53 virus strain (results not shown). Under the conditions of these experiments, M1 and NS1 migrated close to each other, so that additional resolution of immunoprecipitates obtained with mouse anti-M1 monoclonal antibodies was attempted by SDS-PAGE (Fig. 1A).

Relative amounts of viral proteins synthesized in MDCK and Vero cells infected with influenza A virus are reported in Table 3. At 5 h postinfection, the two cell types contained similar proportions of NP, M2, and NS2, while Vero cells contained approximately 10% more HA and 10% less M1/NS1 than did MDCK cells. To determine which protein (M1 or NS1) was underproduced in Vero cells, we also used anti-M1 monoclonal antibodies to analyze the material immunoprecipitated from infected cells. The results (not shown) demonstrated a slightly smaller amount of M1 protein in Vero cells (9.2 versus 14.7% in MDCK cells). As shown in Fig. 1B and Table 3, the pattern of protein synthesis and proportions of the HA, NP, M1, and NS1 proteins were similar in Vero and MDCK cells infected with influenza B/Ann Arbor/1/86 virus. However, in both cell systems, there were differences between the parental and Vero-adapted viruses, in that one polypeptide of the adapted strain showed slower electrophoretic migration on SDS-PAGE; the migration of the HA band in radioimmunoprecipitations and results with molecular weight markers led us to tentatively identify this more slowly migrating protein as NS1.

Ultrastructural features of virus-infected Vero cells. To determine if influenza virus-infected Vero cells show the same morphological changes as other polarized epithelial cells, we studied the ultrastructural features of these cells in comparison with MDCK cells. Both types of cells showed nuclear and cytoplasmic inclusions typical of influenza virus-infected cells, as well as numerous budding virions (Fig. 2A and B). As in MDCK cells, influenza A and B virions were released from the apical surface of Vero cells, a feature typical of epithelial cells infected with influenza virus. The budding virions in both Vero and MDCK cells appeared to be mainly filamentous. A portion of influenza A and B virus-infected cells in both systems showed cytopathological changes indicative of apoptosis (Fig. 2C to F) (30). The nuclear changes consisted of blebbing of the nuclear envelope and condensation of the chromatin. The cytoplasmic changes consisted of extensive vacuolation, blebbing, and vesiculation of the plasma membrane to form "apoptotic bodies."

To confirm that our electron-microscopic observations were indeed consistent with cell apoptotic changes, we examined infected Vero and MDCK cells by an assay that detects fragmented DNA in the cells. The results were positive for 20 to 30% of the infected cells but for none of the uninfected cells (data not shown). This range of positivity may be underestimated, because many positive cells could have detached from the substratum during the extensive washing required by these procedures. In certain cells, the fluorescent label was clearly seen over spherical masses within the nucleus, which may represent condensed masses of degraded DNA. Thus, a substan-

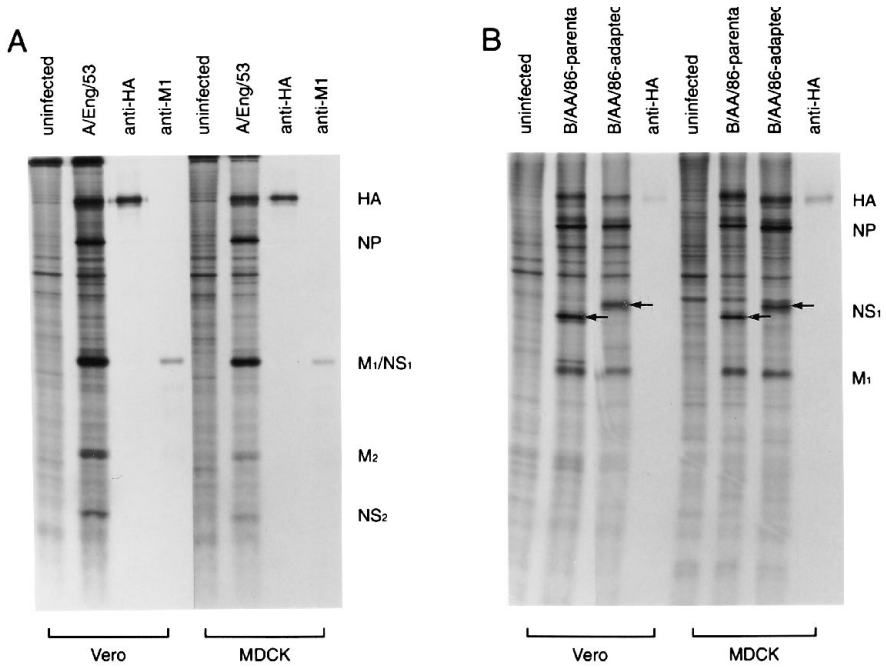

FIG. 1. Protein synthesis in Vero and MDCK cells infected with influenza A/England/1/53 (H1N1) virus (A) or influenza B/Ann Arbor/1/86 virus (B). Vero or MDCK cells were infected with either influenza A or B virus at an MOI of ~30 PFU per cell. After adsorption for 1 h at 37°C, the cells were washed and incubated for 5 h (for influenza A virus) or 7 h (for influenza B virus). Thereafter, the cells were radioactively labeled with Tran [^{35}S]methionine/cysteine (100 μCi/ml) for 2 h at 37°C. The cells were washed, lysed, and immunoprecipitated with specific monoclonal antibodies against HA, NP, M1, NS1, and M2 proteins. The uninfected cells, cells infected with either influenza A virus (A) or influenza B virus (B), and precipitates were analyzed by SDS-PAGE. The positions of viral proteins HA, NP, M1, NS1, M2, and NS2 are marked. The arrows show the NS1 polypeptide of influenza B/Ann Arbor/1/86 virus.

tial portion of infected Vero and MDCK cells undergo endonucleolytic cleavage of DNA—a feature typically seen in other types of cells infected with influenza virus (7, 28).

DISCUSSION

Several lines of evidence from the present study support the use of Vero cells as a host system for cultivation of influenza A and B viruses for vaccine production: (i) efficiency of primary virus isolation and replication to high infectivity titers; (ii) genetic stability of the HA molecule, with maintenance of antigenic properties characteristic of viruses derived from humans; and (iii) similarities in the pattern of protein synthesis and morphological changes between virus-infected Vero and MDCK cells. Previous attempts to grow influenza viruses in Vero cells were mainly unsuccessful (2, 18, 29), with only occasional cultures yielding detectable HA. Moreover, when Vero cells were infected with influenza A/fowl plague/Rostock/34 (FPV, H7N1) virus, an abortive infection was found, very little infectious virus was released, and its spread was greatly impeded (15). It is now known that Vero cells rapidly destroy exogenous trypsin (12), limiting the replication of influenza viruses with a noncleavable HA to a single cycle. We demonstrate here with numerous strains tested that the stepwise addition of trypsin can circumvent this problem and ensure multicycle replication comparable in all respects to that seen with both human influenza A and B viruses in MDCK cells.

We also show that the HA1 region of influenza B viruses isolated and passaged in Vero cells is indistinguishable from that of their MDCK-grown counterparts, supporting the conclusion that Vero cells do not select host cell-mediated HA variants. Similar correlations were reported by Katz and Webster (11) for the HAs of influenza A (H3N2) viruses isolated in LLC-MK2 and primary guinea pig kidney cells and those of

TABLE 3. Relative amounts of influenza A and B viral proteins synthesized in Vero and MDCK cells[a]

A/England/1/53 (H1N1)			B/Ann Arbor/1/86		
Protein	% of protein synthesized in:		Protein	% of protein synthesized in:	
	Vero	MDCK		Vero	MDCK
HA	32.6	22.1	HA	19.5	22.6
NP	34.1	33.2	NP	38.9	43.2
M1/NS1	21.8	31.4	M1	16.4	13.8
M2	5.8	6.9	NS1	25.2	20.4
NS2	5.7	6.4	NS2	ND[b]	ND

[a] The percentage of each influenza virus protein was calculated according to the intensity of the band on an SDS-PAGE gel (15% polyacrylamide); the intensity of all bands was taken as 100%.
[b] ND, not done.

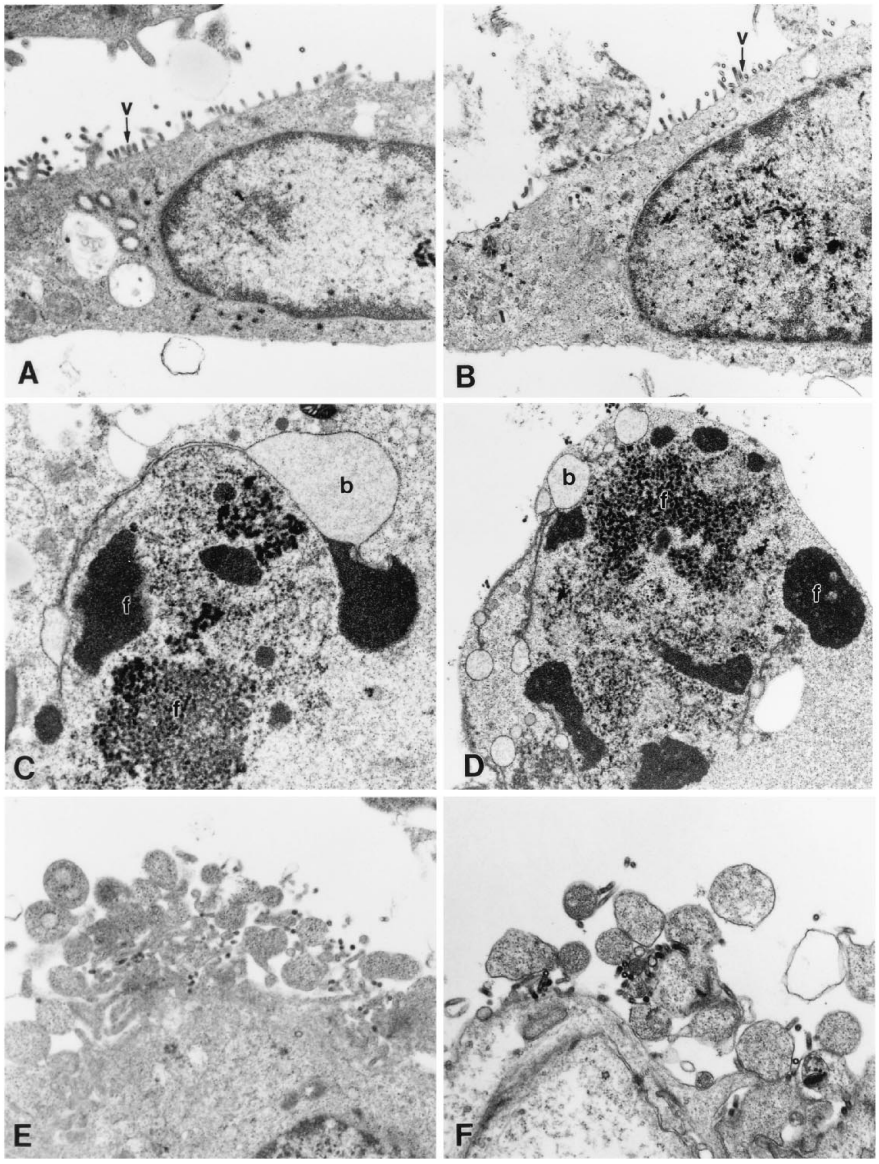

FIG. 2. Electron micrographs showing influenza A virus-infected cells. The virions bud from the apical surface of MDCK cells (A) and Vero cells (B). The nuclear breakdown (C and D) and cytoplasmic blebbing (E and F) typical of apoptotic cells is also observed with MDCK cells (C and E) and Vero cells (D and F). v, virions; b, blebbing of nuclear envelope; f, fragmented nuclei. Magnification, ×9,250.

MDCK cell-grown viruses isolated from the same patient. The absence of host cell-specific modifications of the HA during primary isolation and subsequent passage in Vero cells is a critical requirement in validation of this cultivation system for the production of human influenza vaccines and diagnostic reagents. Alteration in the structure of the HA molecule of influenza viruses grown in eggs or mammalian cells can have a dramatic effect on the antigenicity and immunogenicity of virus (9, 23). Amino acid substitutions at positions 196 to 198 near the tip of the HA molecule of influenza B viruses is known to be involved in host cell-mediated mutations (22, 23). Further, the loss of a potential glycosylation site by egg-grown viruses was associated with alterations in binding of mono- and polyclonal antibodies to the HA molecule (19) and loss of both infectivity and virulence for volunteers (20, 31). Thus, our results on antigenic and nucleotide sequence similarities between the HAs of Vero- and MDCK-grown influenza B viruses provide reassurance against the selection of undesirable variants.

Receptor specificity is an important mechanism governing the susceptibility of cells to virus infection. In the absence of the proper sialic acid receptors, influenza viruses may be unable to bind to the cell surface, thus eliminating the opportunity for productive infection. Although Vero cells bore a relatively low level of the NeuAc α2,6 Gal linkage by comparison with MDCK cells, this relative abundance did not appear to affect their susceptibility to either influenza A or B virus (Table 2). This finding raises the possibility that linkages other than NeuAc α2,3 Gal and NeuAc α2,6 Gal are involved in the attachment of influenza viruses to host cells.

Effective virus replication depends on specific cellular requirements, including the synthesis, transport, and processing of viral proteins needed to produce infectious virus. In the present study, the amounts of HA and M1/NS1 synthesized in

Vero and MDCK cells infected with influenza A virus differed by an estimated 10%. This difference did not affect the virus yield. This observation is consistent with data reported by Nakamura and Homma (18) showing that the synthesis of M protein is selectively inhibited in Vero cells infected with influenza B/Lee/40 virus. By contrast, overproduction of M2 and NS2 proteins in Vero cells infected with A/FPV (H7N1) was recently described by Lau and Scholtissek (15). In any event, the amount of M1 produced in Vero cells seems to be sufficient to facilitate the nucleocytoplasmic transport of the nucleocapsid and the production of infectious virus. Studies on protein synthesis of influenza B/Ann Arbor/1/86 virus in Vero and MDCK cells did show differences between the parental and adapted strains. On the basis of immunoprecipitation and molecular weight markers, this protein has been tentatively identified as NS1.

Thus, there are differences in the replication of influenza A and B viruses in eggs and mammalian cells, and further studies are needed to resolve the molecular basis of these differences. However, the main focus of this study is the ability of influenza A and B viruses to undergo productive replication in Vero cells and antigenic stability of the virus. The molecular mechanisms of influenza virus adaptation to Vero cells are beyond the scope of this study.

Ultrastructural examination of influenza virus-infected Vero cells revealed morphological changes similar to those observed in MDCK cells. It is interesting that both cell lines produce predominantly filamentous viruses, which may explain the detection of lower HA levels than might be expected from the high infectivity rates associated with these host cell systems. Although influenza virus can induce apoptosis in other cell lines (7, 28) the observations reported here provide the first morphological and cytochemical evidence of this effect in Vero cells.

The MDCK cell line has been widely touted as the optimal mammalian cell system for the isolation and growth of influenza viruses, but it has not yet been licensed for use in vaccine production. A report that MDCK cells can induce tumors in nude mice (17a) raises questions about the suitability of this system for the production of live influenza virus vaccines. Thus, the Vero cell line offers an attractive alternative for the cultivation of influenza A and B viruses. This system is already in use for the production of human virus vaccines and could readily be adapted to influenza viruses should a new pandemic strain appear.

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