Evaluation of Three Cell Culture Systems as Substrates for Influenza Virus Assay

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The propagation of various influenza virus strains in primary rhesus monkey kidney (RMK), primary hamster kidney (HK), and the MDCK-USD canine kidney (CK) cell cultures was compared. Virus-infected cultures were examined for cytopathic effect (CPE) and by performing the hemadsorption (HAd) technique. The highest HAd titers were found most often in HK, followed by RMK and CK. However, the CK cell line provided the best substrate for detecting CPE. Although influenza B strains tended to grow to higher titers, these strains produced less CPE than did the A strains.

Previous studies have described the replication and cytopathogenic effects (CPE) of influenza virus in primary and continuous cell cultures of human and animal origin (1–3, 4–6, 8). Although primary rhesus monkey kidney (RMK) cell cultures have been used extensively for influenza virus isolation and neutralization tests, problems such as adventitious hemadsorbing agents and lack of consistency among different cell lots have plagued its use (unpublished data). Many have considered using continuous cell lines; however the susceptibility of most cell lines to influenza virus infection is low. Two cell cultures, namely primary hamster kidney (HK) and MDCK-USD canine kidney (CK) continuous cell line, have shown promise as substrates for propagating influenza viruses. This report describes our studies comparing the growth of a variety of influenza virus strains in eggs and in primary RMK, primary HK, and the MDCK-USD canine cell cultures.

MATERIALS AND METHODS

Viruses. All virus strains were part of the reagent collection maintained in our laboratory. Table 1 lists the strains used in this study and their passage histories. The egg- and RMK-adapted pools of a particular strain were titrated concurrently.

Cell cultures. Primary RMK cells were grown in Hanks balanced salt solution with 0.5% lactalbumin hydrolysate. Primary HK cells were cultured from kidneys removed from 18-day-old Syrian hamsters using minimum essential medium with Hanks salt base (MEMH) and 5% fetal calf serum as growth medium. The MDCK-USD canine kidney cell line (1) was grown in MEMH with 10% fetal calf serum. All cells were grown in Pyrex screw-cap tubes (16 by 125 mm) at 37 C and maintained at 32 C. Maintenance medium consisted of Hanks balanced salt solution. In the case of RMK cell cultures, 0.2% SV-5 antiserum was added to the maintenance medium.

Infectivity titrations. Titrations were simultaneously performed in chicken embryos and in the three cell culture systems.

Each virus strain was titrated in 10-day-old chicken embryos which received 0.1 ml of serial 10-fold dilutions into the allantoic sac. Six eggs were used at each dilution point. After incubation of the subtype A strains at 32 C for 48 hr and the subtype B strains for 64 hr, titration end points were determined by checking for the presence of hemagglutinin in the allantoic fluid.

After the growth medium was removed from the cell cultures, 0.1-ml samples of the same virus dilutions inoculated into eggs were also inoculated on the cell monolayers. Four tubes were used at each dilution point. After adsorption for 1 hr, 1.0 ml of maintenance medium was added and the tubes were subsequently incubated at 32 C for 72 hr. The virus-infected cell cultures were first examined for the presence of CPE. In addition, the presence of virus was also determined by hemadsorption tests performed according to the method of Shelokov et al. (9).

Calculations. All end points were calculated by the Kärber formula (7).

RESULTS

The growth of some influenza virus strains in chicken embryos and in primary RMK, primary HK, and a continuous line of CK cells is shown in Table 1. Virus growth of all strains, including the RMK-adapted strains, was consistently highest in the chicken embryo allantoic sac.

Concerning the growth of A strains in cell culture, the highest titers, as determined by hemad-
### Table 1. Comparative assay in tissue culture and chicken embryos of influenza virus growth and cytopathogenic effect

| Influenza strain       | Passage history | Egg ID<sub>50</sub> | 1<sup>°</sup>RK<sup>60</sup> | 1<sup>°</sup>HK<sup>60</sup> | CK<sup>60</sup> | Avg<sup>b</sup> |
|------------------------|---------------|--------------------|-----------------|-----------------|-------------|-------------|
|                        |               |                    | CPE<sub>50</sub> | HAd<sub>50</sub> | CPE<sub>50</sub> | HAd<sub>50</sub> | CPE<sub>50</sub> | HAd<sub>50</sub> |
| A/PR/8/34              | E/6, RMK/3    | 5.5<sup>a</sup>    | 0.5       | 3.5       | 1.8       | 4.8       | 2.5       | 2.8       | 1.6       | 3.7       |
| A/PR/8/34              | E/6           | 4.5                | 1.5       | 2.5       | 0.5       | 4.5       | 2.5       | 2.5       | 1.5       | 3.2       |
| A/Swine/1976/31        | M/38, E/40,   | 5.3                | 1.5       | 3.5       | 4.0       | 5.3       | 1.5       | 2.8       | 2.3       | 3.9       |
|                       | RMK/3         |                    |            |            |            |            |            |            |            |            |
| A/Swine/1976/31        | E/40          | 7.5                | 1.5       | 6.5       | 2.5       | 6.5       | 3.3       | 4.5       | 2.4       | 5.9       |
| Avg<sup>c</sup>        |               | 5.7                | 1.3       | 4.0       | 2.2       | 5.3       | 2.4       | 3.2       | 1.9       | 4.2       |
| A1/FM/1/47             | E/2           | 7.8                | 0.0       | 3.8       | 0.5       | 5.8       | 2.5       | 3.5       | 1.0       | 4.4       |
| A1/FLW/1/52            | E/13          | 8.3                | 1.5       | 6.3       | 0.5       | 5.5       | 3.5       | 4.5       | 2.2       | 5.4       |
| A1/AA/1/57             | E/6, RMK/3    | 6.3                | 0.5       | 3.5       | 1.5       | 4.8       | 2.5       | 2.8       | 1.5       | 3.7       |
| A1/AA/1/57             | E/7           | 6.3                | 0.5       | 2.5       | 1.5       | 3.8       | 2.5       | 2.8       | 1.5       | 3.0       |
| Avg<sup>c</sup>        |               | 7.2                | 0.6       | 4.0       | 1.0       | 5.0       | 2.8       | 3.4       | 1.6       | 4.1       |
| A2/Jap/170/62          | E/9, RMK/3    | 5.5                | 1.5       | 4.5       | 1.5       | 4.5       | 1.5       | 2.8       | 1.5       | 3.9       |
| A2/Jap/170/62          | E/7           | 7.0                | 1.5       | 5.0       | 2.5       | 3.5       | 1.5       | 5.5       | 1.8       | 4.7       |
| A2/AA/7/67             | E/6, RMK/3    | 5.5                | 0.5       | 3.5       | 1.5       | 3.3       | 2.5       | 2.5       | 1.5       | 3.1       |
| A2/AA/7/67             | E/8           | 6.3                | 0.5       | 3.5       | 1.0       | 5.5       | 2.5       | 3.5       | 1.3       | 4.2       |
| A2/Aichi/2/68          | E/6, RMK/3    | 4.8                | 0.0       | 2.5       | 0.5       | 2.5       | 2.5       | 2.5       | 1.0       | 2.5       |
| A2/Aichi/2/68          | E/8           | 5.0                | 0.0       | 1.5       | 0.5       | 2.5       | 2.0       | 2.0       | 0.8       | 2.0       |
| A2/Rockville/1/65      | RMK/7         | 3.9                | 0.5       | 3.5       | 1.5       | 4.5       | 1.5       | 3.5       | 2.8       | 1.8       |
| A2/Rockville/1/65      | E/7           | 5.3                | 0.0       | 3.3       | 0.0       | 3.5       | 1.5       | 2.5       | 0.5       | 3.1       |
| A2/HK/1/68             | RMK/3         | 6.0                | 0.0       | 3.5       | 1.0       | 3.6       | 2.1       | 3.1       | 1.2       | 3.4       |
| Avg<sup>c</sup>        |               | 5.4                | 0.6       | 3.5       | 1.0       | 3.6       | 2.1       | 3.1       | 1.2       | 3.4       |
| B/Lee/40               | RMK/3         | 5.3                | 0.5       | 2.5       | 0.5       | 3.3       | 1.5       | 2.5       | 0.8       | 2.8       |
| B/Lee/40               | E/3           | 8.0                | 0.5       | 5.5       | 0.5       | 5.5       | 1.5       | 4.5       | 0.8       | 5.2       |
| B/Md/1/59              | RMK/3         | 4.0                | 0.0       | 4.3       | 0.0       | 4.5       | 1.5       | 4.0       | 0.5       | 4.3       |
| B/Md/1/59              | E/10          | 7.3                | 1.5       | 5.5       | 0.0       | 5.5       | 3.5       | 6.5       | 1.7       | 5.8       |
| B/Mass/3/66            | E/6, RMK/3    | 5.5                | 0.8       | 4.0       | 0.5       | 4.3       | 0.5       | 4.5       | 0.6       | 4.3       |
| B/Mass/3/66            | E/8           | 5.3                | 0.0       | 4.3       | 0.0       | 3.8       | 0.5       | 4.8       | 0.2       | 4.3       |
| Avg<sup>c</sup>        |               | 5.9                | 0.6       | 4.4       | 0.3       | 4.5       | 1.5       | 4.5       | 0.8       | 4.5       |
| Avg<sup>d</sup>        |               | 6.2                | 0.7       | 4.0       | 1.1       | 4.6       | 2.2       | 3.8       | 1.4       | 4.4       |

<sup>a</sup> Key: 1<sup>°</sup>RKM, primary rhesus monkey kidney; 1<sup>°</sup>HK, primary hamster kidney; CK, the MDCK-USD-clone "E" cell line; CPE<sub>50</sub>, 50% infectious end point as determined by cytopathic effect; HAd<sub>50</sub>, 50% infectious end point as determined by hemadsorption; ID<sub>50</sub>, 50% infectious end point; E, egg; M, mouse.

<sup>b</sup> Average endpoint in the three cell cultures.

<sup>c</sup> Average of the column.

<sup>d</sup> Average of the entire column.

<sup>e</sup> All titers are expressed as log<sub>10</sub> values per 0.1 ml.

Sorption, were achieved in primary HK. B strains appeared to grow equally well in all three cell culture systems. The hemadsorption technique was more sensitive than CPE for demonstrating the presence of virus. It was easier to detect CPE in the CK than in the other two cell culture systems. Although the B strains grew equally well in all three cell culture systems, the A strains consistently produced more CPE.

Considering the overall growth of all virus strains in the three cell culture systems, as determined by hemadsorption, the highest titers were achieved in primary HK (70%); primary RMK (30%) and the CK cell line (22%) averaged lower. When growth is evaluated by the less-sensitive procedure of observing for CPE, the highest titers were found in the CK line (87%); primary HK (13%) and RMK (8.7%) were less sensitive. (Percentages were calculated by dividing the number
of virus strains which titered highest in each of the cell cultures by the total number of virus strains employed and multiplying by 100. The total of percentages is greater than 100 because on several occasions a strain would have an equally high titer in 2 of the 3 cell cultures used.)

DISCUSSION

We concluded from our study of three cell culture systems that primary HK was consistently the best and most sensitive indicator for influenza virus multiplication. Primary RMK is utilized extensively for influenza virus isolation and neutralization tests. However, there are disadvantages in using RMK cell cultures, e.g., the presence of adventitious hemadsorbing agents and the lack of consistency among different cell lots. Primary HK cell cultures would be superior because they are more sensitive to influenza virus infection and because no latent viruses, which would interfere with the detection of influenza, have been demonstrated thus far in this cell culture. The lack of latent hemadsorbing viruses in primary HK cells precludes the necessity to add such variables as hyperimmune serum as is required when primary RMK cells are to be used for such work.

According to S. E. Grossberg (personal communication), an important factor which must be emphasized is that kidney cultures prepared from hamsters which are younger than 18 days tend to be more resistant to growth of influenza virus. Grossberg advised obtaining kidneys from hamsters between the ages of 18 and 35 days.

Although the growth of only one strain was higher in HK cell cultures than in chicken embryos, the overall growth in HK could be considered quite favorable, especially in light of the fact that none of the strains was adapted to grow in HK cells. It would probably be feasible and advantageous to prepare inactivated cell culture-derived influenza virus vaccine.

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