Growth of Respiratory Syncytial Virus in Suspension Cell Culture

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Respiratory syncytial virus, Burnett strain, adsorbed efficiently and grew to high titers in suspension cultures of HEp-2 and MA-160 cells. Our results compared favorably with previous experience with the growth of respiratory syncytial virus in monolayer cell cultures. The use of suspension cell cultures provides a convenient and simple procedure for producing high-titering respiratory syncytial virus pools.

MATERIALS AND METHODS

Virus. The RS virus, Burnett strain, used for all experiments had undergone four passages in primary human embryonic kidney cells and nine passages in HEp-2 cell cultures since isolation. A common work pool, stored at −70°C and used throughout these studies, had an infectious titer at the time of preparation of 10^3.4 TCID₅₀ per 1.0 ml, as calculated by the Kärber method (7). The titer remained stable throughout the duration of these studies.

Suspension cultures. Suspension cultures of HEp-2 cells were obtained from Flow Laboratories, Rockville, Md.; suspension cultures of MA-160, a continuously propagated line of human heteroploid cells derived from benign human prostate, were kindly provided by Microbiological Associates, Bethesda, Md. The medium used was Eagles’ minimum essential medium for suspension cultures, supplemented with 0.03% glutamine, 100 units of penicillin G per ml, 100 μg of streptomycin sulfate per ml, and 10 or 5% fetal calf serum for HEp-2 and MA-160 cells, respectively. After centrifugation (200 × g for 15 min), a previously determined number of cells was brought into contact with a volume of virus inoculum sufficient to supply the required virus-to-cell multiplicity. Although experiments were performed with virus-to-cell multiplicity ratios in a range of approximately 1:100 to 5:1, only those experiments with a multiplicity ratio of 5:1 will be included in the discussion below. This mixture was gently swirled every 15 min at 37°C while fresh medium was added at 30-min intervals, so as to provide for the final volume at 2 hr after initial virus contact. The final culture volume provided a cell density of from 2 × 10^6 to 3 × 10^6 cells/ml. All cultures were continuously agitated and kept open to the air at 37°C. Cell counts were performed at times of viral harvest by using trypan blue exclusion as a measure of cell viability. Parallel control cultures were similarly handled.

Virus harvest and assay techniques. All infected and control cultures were sedimented and refed with an identical volume of fresh medium at 12 and 24 hr and daily thereafter until no viable cells remained in infected cultures. Samples of both fluid and cells and cell-free supernatant fluids were collected and stored at −70°C until assayed for infectious virus. All specimens were tested within 2 weeks of harvest.

Infectious virus was assayed in monolayer cultures of the MA-104 embryonic rhesus monkey kidney cell line. This has proved to be a convenient and reproducible assay system because MA-104 cell cultures require few media changes and syncytia formation is quite prominent in this line. Infectious titers were expressed as TCID₅₀ per 0.1 ml as calculated by the Kärber method (7).

RESULTS AND DISCUSSION

Adsorption. Cell-free supernatant specimens of HEp-2 infected cultures were collected as described above at 2-hr intervals beginning at 0 hr and extending through 12 hr after infection. Since it was previously determined that there was no virus production in the initial 12 hr, the virus remaining in the supernatant fluid at periods during that interval represented the difference between the initial virus input and the fraction which either had adsorbed to the cells or had been...
thermoinactivated. Figure 1 depicts both the plot obtained from experimental data and that corrected for RS virus thermolability, assuming a 10-fold exponential decline every 24 hr at 37 C. The latter observation has been made by previous investigators (1, 4). The adsorption of RS virus to HEp-2 cells in suspension was slow but efficient. Greater than 90% of input virus was adsorbed by 4 to 6 hr, with more than 98% absorbed by 12 hr.

**Virus growth.** Figure 2 shows a composite curve of six identical experiments representing a single-cycle growth curve of RS virus in HEp-2 cell cultures. The input virus-to-cell multiplicity was approximately 5:1 for these cultures. We observed a latent period of 12 hr, followed by an exponential growth phase. After reaching a slight plateau at 24 to 34 hr, there occurred further exponential growth until maximal titers were reached at 48 hr after infection. It can be assumed that this represented a single-cycle growth curve, as virtually 100% of the cells fluoresced specifically with RS virus hyperimmune serum at 10 hr postinfection, a time when presumably only initial cell-virus contact occurred. This judgment is based on the previous finding that RS virus fluorescence in HEp-2 cells began at about 10 to 12 hr after infection (6). Infection was lytic with complete cellular destruction by 96 hr. At the peak of virus production, most of the infectious virus remained cell-associated, as assayed in our system, but we saw eventual extracellular release upon death and lysis of cell.

The growth of RS virus in MA-160 suspension cell cultures was similar to the growth in HEp-2. Virtually 100% of the virus was adsorbed to MA-160 cells by 3 hr, and peak virus titers which occurred 5 days after infection were similar to the peak titers achieved in HEp-2 suspension cultures. Again, most of the virus remained cell-associated. Complete cell death occurred by 5 to 6 days.

We have demonstrated that RS virus can replicate efficiently in a suspension culture system. Our figures of 98% adsorption in HEp-2 suspension cultures are higher than the 80 to 90% efficiency reported by previous investigators (2, 3) for adsorption in HEp-2 monolayer cultures. Peak infectious titers compare favorably with the highest titers observed in HEp-2 monolayers. The data for suspension cultures closely parallel those for HEp-2 monolayers, with latent periods of 12 to 16 hr and peak virus production at 45 to 50 hr (2, 5, 6, 8). Continuously agitated suspension cultures might provide a better alternative to monolayers for the production of large amounts of high-titered RS virus pool and complement-fixing antigen.

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