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Growth Characteristics of Two Rhinovirus Strains in WI-26 and Monkey Kidney Cells

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ABSTRACT

HAFF, R. F. (Smith Kline & French Laboratories, Philadelphia, Pa.), B. WOHLSEN, E. E. FORCE, AND R. C. STEWART. Growth characteristics of two rhinovirus strains in WI-26 and monkey kidney cells. J. Bacteriol. **91**:2339–2342. 1966.—Viruses with 1059 and HGP serotype and with human and monkey host range characteristics, respectively, were employed. Adsorption kinetics of the 1059 and HGP strains to WI-26 cells, and HGP to Green African monkey kidney cells (MKC), were similar. Fifty per cent of the virus was adsorbed to cell monolayers within 10 min; adsorption was essentially complete by 2 hr. The 1059 strain failed to adsorb to MKC, at least to an appreciable extent. Lack of receptors for adsorption of 1059 accounts for the inability of this cell to support multiplication of the virus. It is probable that MKC are refractory to infection with other H strains of rhinovirus for the same reason. Single-step multiplication cycles have been described for the HGP strain in WI-26 and MKC cultures and for the 1059 strain in WI-26 cells. In both cells, HGP exhibited a latent period of 7 hr. Increase of intracellular and cell-associated virus appeared somewhat prior to that of extracellular virus. Maximal titers were attained by 9 to 10 hr. In contrast, initial increase of 1059 in WI-26 cells occurred after 10 hr. Titer rose to peak level 15 hr after infection. Yield of 1059 in WI-26 cells was also fivefold lower than that of HGP in either cell system.

Laboratory studies with rhinoviruses have been largely directed toward isolation, titration, identification, and classification of agents comprising this group. Little attention has been devoted to characteristics of their adsorption to, or multiplication in, cultured cells. To provide such information, comparative studies were carried out with virus strains possessing 1059 (4) and HGP (8) serotype, and with human and monkey host range characteristics (7), respectively, as determined by preliminary tube titration. Cells from human embryonic lung (strain WI-26) and African green monkey kidney (MKC) were employed.

MATERIALS AND METHODS

Viruses. The 1059 strain was obtained from the American Type Culture Collection. After isolation in cultures of human embryonic kidney, the virus was serially passed twice in this cell line, once in WI-26 cells, 10 times in KB cells, and 3 times in WI-26 cells. An HGP strain was provided by D. A. J. Tyrrell. This virus was isolated in cultures of human embryonic kidney, then serially passed five times in the same cell line, six times in monkey kidney, two times in HEp-2, two times in monkey kidney, and finally two times in WI-26 cells. For brevity, these virus strains will subsequently be designated 1059 and HGP. Viruses were propagated in tube cultures of WI-26 cells at 34 C on a roller drum operating at 12 rev/hr in medium composed of Eagle's minimal essential medium (1.4 g per liter of $NaHCO_3$), Eagle's nonessential amino acids, 100 μg/ml of neomycin, 100 units per ml of penicillin G, 100 μg/ml of streptomycin sulfate, and 10% fetal calf serum. Stock viruses consisted of culture medium harvested 3 to 5 days after inoculation with $10^{2.5}$ $TCID_{50}$ of virus when viral cytopathic effect (CPE) was essentially complete. The titer of 1059 stock was $10^{5.0}$ $TCID_{50}$/ml and that of HGP stock was $10^{5.2}$ $TCID_{50}$/ml.

Cell cultures. WI-26 cells were obtained in suspension from BBL. Tube cultures for virus titration were established with 6×10^4 cells in 1 ml of medium. Cultures were incubated at 34 C for 5 to 6 days in a stationary position. Medium was replenished on day 3. Tube cultures for growth curves were inoculated with 3×10^4 cells; medium was also changed on the 3rd day, and cultures were used on day 4, when each yielded about 1.3×10^5 cells on trypsinization. Petri dishes (100 mm; Falcon Plastics, Los Angeles, Calif.) were seeded with 2×10^6 cells in 10 ml of medium. After incubation in an atmosphere of 3% carbon dioxide and air at 37 C for 4 days, with medium change on day 3, confluent monolayers had formed

which were ready for use. Tube cultures of MKC, containing approximately 1.4×10^5 cells, were obtained from BBL. Suspensions of MKC obtained from the same source were used to establish monolayer cultures in petri dishes. Cells (6×10^6) were inoculated into each dish in 10 ml of medium. Cultures were refed after incubation for 4 days at 37 C and used 3 days later.

Tube titration. Each of four WI-26 cultures was inoculated with 1 ml of serial 10-fold dilutions of virus in medium. Cultures were rolled at 34 C. The $TCID_{50}$ titer of virus was determined on the basis of viral CPE 6 days after inoculation with 1059 and after 5 days with HGP. Although cultures exhibiting CPE at these periods of incubation were further destroyed with time, CPE was rarely first observed at a later interval.

Plaque titration. Foci of infection in petri dish cultures were produced as follows. After viral adsorption (see below), the inoculum was removed and cultures were washed with 3 ml of phosphate-buffered saline (PBS), *p*H 7.6, containing 0.1% bovine serum albumin (PBS-albumin). WI-26 cultures were overlaid with 6 ml of medium supplemented with 1% agar (Difco) and incubated at 37 C. An additional 6 ml of the same medium was added on day 3. After 7 days, 3 ml of [2(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl] tetrazolium chloride (INT) was added (1), and plaques were counted 24 hr later. MKC cultures were overlaid with 6 ml of medium containing agar. After incubation for 4 days at 37 C, an additional 6 ml of the same medium supplemented with a 1:40,000 dilution of neutral red was added. Plaques were counted after 7 days.

Adsorption experiment. Petri dish cultures were washed with 3 ml of PBS-albumin. A 1-ml amount of virus diluted in PBS-albumin to provide a maximum of 50 to 150 plaques was added to each culture. After different intervals, cultures were washed and incubated, as described above, to permit development of plaques. The relationship of plaque count to interval of contact of virus inoculum with the culture was employed to describe the kinetics of viral adsorption with all virus-cell combinations except 1059 and MKC. No plaques were produced by 1059 in these cells. In the latter instance, therefore, virus inoculum was removed as completely as possible from MKC monolayers after different periods of contact. The inoculum from each MKC culture was placed on a monolayer culture of WI-26 cells for 1 hr to permit virus adsorption. Virus was then assayed by plaque titration. Three cultures were used for each interval of adsorption. Results are averages of data from two experiments.

Growth curves. A 0.5-ml amount of medium, containing 5×10^4 $TCID_{50}$ of virus (multiplicity of infection = 0.4), was added to each of a series of tube cultures. Cultures were incubated at 34 C in a stationary position. After 1 hr (considered 1 hr postinfection), medium was removed and cultures were washed three times with 5 ml of medium. A 1-ml amount of fresh medium was added, and cultures were reincubated at 34 C on a roller drum. Infected cultures were assayed by tube titration for extracellular, cell-associated, and intracellular virus after different intervals, with use of pooled material from four replicate cultures at each period of time. Results presented are mean values from two experiments.

Extracellular virus. Medium removed from the cultures was employed.

Cell-associated virus. Cultures were washed with 5 ml of medium. Cells were suspended by treatment of each culture with 0.5 ml of 0.04% trypsin ($2 \times$ crystallized; salt-free) in PBS at *p*H 8.0 for 5 min at 23 C. An equal volume of 0.06% soybean trypsin inhibitor in PBS-albumin at *p*H 7.6 was then added. Virus remaining in the medium after sedimenting cells by centrifugation at $800 \times g$ for 7 min was assayed.

Intracellular virus. Cells were washed by centrifugation in 1 ml of medium per culture. Cells were resuspended in the same volume of medium and disrupted by three cycles of freezing and thawing between 37 and −70 C. Virus remaining in suspension after removal of cell debris by centrifugation at $800 \times g$ for 7 min was assayed.

RESULTS

The kinetics of rhinovirus adsorption to cell monolayers at 23 C are illustrated in Fig. 1. The characteristics of adsorption of the 1059 and HGP strains to WI-26 cells, and HGP to MKC, were similar. Approximately 50% of the adsorption observed within the 2-hr period of the experiment occurred during the initial 10-min interval of contact. Thereafter, adsorption was

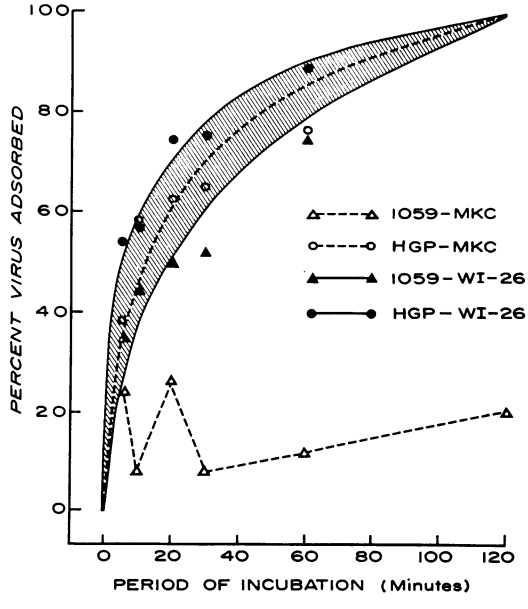

FIG. 1. *Kinetics of rhinovirus adsorption to cell monolayers at 23 C. Amount adsorbed in 2 hr is designated 100% for all combinations but 1059-MKC; for the latter system, 100% is that amount added to cultures.*

progressively slower. In contrast with the above virus-cell combinations, no more 1059 was retained by MKC cultures after incubation for 2 hr than after 5 min, as indicated by titration of unadsorbed virus in WI-26 cells. After 2 hr, 80% of that added was recovered.

Multiplication of 1059 and HGP in WI-26 and MKC cultures was examined under conditions permitting essentially synchronous infection of a large proportion of the cells (Fig. 2). Little or no 1059 increase was demonstrable in MKC. Conversely, HGP multiplied readily in this cell type. Increase in intracellular and cell-associated virus was initially detectable 7 hr after infection; extracellular virus showed a somewhat delayed significant rise in titer. Peak virus concentration was attained by 9 to 10 hr. Apparent yield of virus per infected cell was approximately 0.2. The characteristics of HGP multiplication in WI-26 cells were approximately the same as those in MKC. On the other hand, 1059 in WI-26 cells showed a longer latent period (10 versus 7 hr), a slower rate of increase (maximal titer at approximately 15 versus 9 hr), and lower yields of virus per infected cell (0.04 versus 0.2). Also, there was no evidence of a delayed release of virus from the cell.

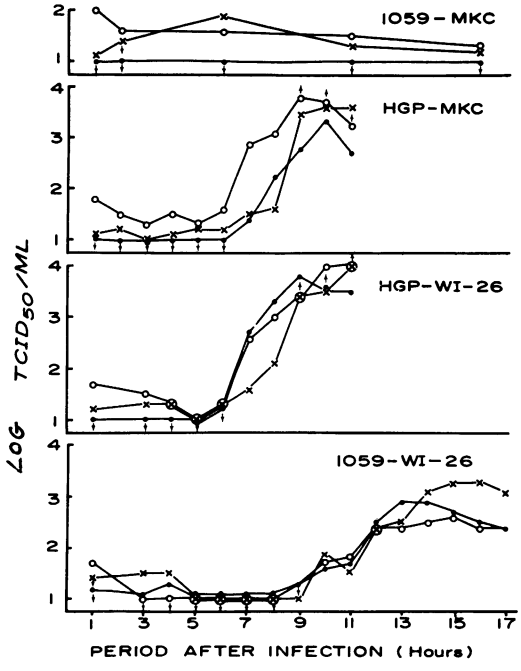

FIG. 2. *Single-step cycles of rhinovirus multiplication in tube cultures rolled at 34 C. Symbols:* ● = *intracellular virus;* ○ = *cell-associated virus;* × = *extracellular virus;* ↑ = >; ↓ = <.

DISCUSSION

The kinetics of rhinovirus adsorption to cells clearly supporting their replication were essentially the same among the virus-cell combinations studied. Similar characteristics of adsorption have also been observed for an HGP strain in secondary cultures of Rhesus monkey kidney (5). It is unlikely that the observed 20% retention of 1059 by MKC cultures resulted from adsorption of virus to these cells. Retention of a constant amount of virus by MKC cultures at each interval of assay probably reflects the fact that the inoculum could not be entirely recovered from the MKC cultures for assay.

Lack of 1059 multiplication in MKC is a logical consequence of the observed inability of virus to adsorb to the cell. This finding is consistent with the conclusion of Holland that cell susceptibility to the closely related enteroviruses is primarily dependent on the presence of specific cell receptors (2, 3). Although unproven, it is probable that MKC are refractory to other H strains of rhinovirus for the same reason.

Rhinovirus multiplication cycles have not been described heretofore, although growth of an HGP strain in MKC has been studied (6). Under "optimal" conditions of infection, Tyrrell demonstrated an initial 12-hr increase in titer of intracellular virus and a somewhat later release of newly synthesized virus into the medium. The time of virus increase shown by Tyrrell may not delimit the latent period of the infection, since initial input of virus was relatively low. The observed increase could, therefore, occur after the second cycle, providing a latent period more closely approximating that obtained in the present study with HGP in MKC (7 hr). The observed delay in release of extracellular virus was, however, substantiated. It is evident that the characteristics of rhinovirus multiplication depend on the system employed. Tyrrell (6) demonstrated this fact by modifying conditions of incubation of HGP-infected MKC. In the present study, the pattern of 1059 multiplication in WI-26 cells differed in several respects from that of HGP in both WI-26 and MKC cultures. In addition, the production of 1059 in WI-26 cells was approximately fivefold lower than that of HGP in either cell type. Although apparent virus yield per cell was less than 1 in all systems employed, only relative figures are meaningful until the sensitivity of the titration method has been correlated with particle count.

The above studies do not imply that all strains of virus with 1059 and HGP serotype possess similar characteristics of interaction with cells of human and simian origin. The HGP strain

which was used had been previously passed in both monkey kidney and human cells, whereas 1059 had been propagated only in cells from human tissue. Since host range characteristics of viruses are mutable, it is reasonable that passage of 1059 in monkey kidney cells could select a virus strain which interacts differently with this host.

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