HL Cells, a Sensitive Line for the Isolation and Propagation of Respiratory Syncytial Virus[1] (36491)

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Continuous epithelial cell cultures of human origin, such as HeLa or HEp-2 are usually the preferred systems for initial isolation of respiratory syncytial (RS) virus, adenoviruses, herpesvirus hominis and certain coxsackieviruses (1). In our laboratory, we have found another human epithelial cell culture, the HL cell line, to be at least equally sensitive to these and other respiratory viruses and to offer the advantage of a slower growth rate, allowing prolonged observation during primary isolation. The following report presents a comparison of the HL and HEp-2 cell culture systems for viral isolation and propagation.

Materials and Methods. Cell cultures. HL (Human line) cells have been maintained in this laboratory for the past 10 years. They have been found to be a sensitive host for propagation of RS virus (2). In 1964, the line was demonstrated to be heteroploid (modal number of cell chromosomes 73) and human origin was confirmed by fluorescent antibody studies (W. D. Peterson, Jr., Child Research Center of Michigan, Detroit, MI). The source of these cells is unknown.

The strain of HEp-2 cells (3) used was obtained originally from Dr. D. Merchant, W. Alton Jones Cell Science Center, Lake Placid, NY, and has since been maintained in our laboratory.

Media. Media used for HL and HEp-2 cells were those found in this and other laboratories to be optimal for growth and maintenance. The HL cells were grown in Eagle's minimum essential medium in Hanks' salts (HMEM) supplemented with 5% fetal bovine serum; Eagle's minimum essential medium in Earle's salts (EMEM) supplemented with 10% fetal bovine serum was used for HEp-2 cell growth. Both cell lines were maintained with EMEM containing 2% inactivated chicken serum. All media contained a final concentration of 100 units of penicillin, 100 μg of streptomycin and 10 μg of chlortetracycline/ml.

Cell growth and preparation. The HL cells were grown at 37° in 32 oz prescription bottles. The cultures were initiated at a cell count of 4.5–5.0 $\times$ 10^5 in 50 ml of growth medium. The feeding schedule consisted of a 50% growth medium change on the third day after subculturing, followed by a 100% medium change (EMEM with 5% fetal bovine serum) on day 6 or 7 after subculturing. Confluent monolayers were formed by day 10. HL cells grown in this manner were used in preparing tube cultures which were seeded with 9–10 $\times$ 10^4 cells in 1 ml. Following 2–3 days of growth, the cultures were ready for use; cell monolayers were washed once with Hanks' balanced salt solution (HBSS), placed on maintenance medium and incubated at 37° until used.

HEp-2 cells were propagated and maintained in a similar manner. Confluent monolayers were subcultured weekly; new bottle cultures received a 100% maintenance medium charge on day 2 or 3 after subculturing and confluent monolayers were formed by day 5.

Determination of cell growth rates. Growth rates of HL and HEp-2 cells were compared by seeding 2.0 $\times$ 10^4 cells of either line into

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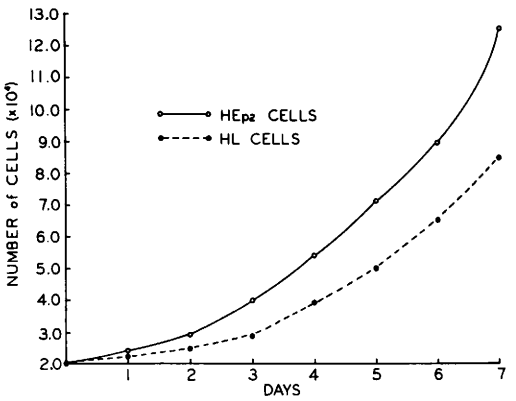

FIG. 1. Growth curves of HL and HEp-2 cell cultures.

2 oz prescription bottles with 5 ml of growth medium. Bottles were incubated at 37° and, at 24 hr intervals, two bottles of both lines were selected at random and cells were counted.

Comparative virus susceptibility of HL and HEp-2 cells. Clinical specimens from which RS virus had previously been isolated had been stored at —70° since collection; the medium consisted of veal infusion broth with 0.5% bovine plasma albumin (4). Reisolations of RS virus were attempted by making a 10^{-1} and 10^{-2} dilution of the specimen in maintenance medium and inoculating 0.2 ml of the original specimen and diluted material each into 2 tubes of both cell lines. The tubes were incubated at 33° on a roller drum and the day of first appearance of definite cytopathic effect (CPE) was noted.

Laboratory strains of various respiratory viruses were inoculated into both HL and HEp-2 cells at 33°. Culture fluids were harvested when maximal CPE was evident and passed serially into fresh culture tubes until maximum CPE occurred within 2–3 days following inoculation. Infectivity titrations were then performed in both cell culture systems; serial tenfold dilutions were prepared in maintenance medium and 4 tubes were inoculated per dilution. Infectivity endpoints were calculated on day 7 by the method of Reed and Muench (5).

Results. Characteristics of HL cell line. HL cells are typically epithelial in morphology; similar, but distinct in appearance to that of HEp-2 cells. They have been propagated for long periods without change in morphologic or growth patterns.

Rate of growth of HL cells and of HEp-2 cells is compared in Fig. 1. The more rapid growth of HEp-2 cells was evident at 24 hr after initiation and the difference increased with time. By 7 days there were 1.5 times as many HEp-2 cells as HL cells. The relatively slow growth rate facilitates maintenance of monolayers over periods of time, without nonspecific cell degeneration.

Growth and isolation of RS virus in HL cells. Infection of the HL cell line with respiratory syncytial (RS) virus produced characteristic cytopathic changes similar to those produced in sensitive strains of HEp-2 cells (6). The cytopathic effect initially consisted of prominent focal areas of vacuolated syncytial cells; these areas progressed and became confluent, usually with thin cytoplasmic strands connecting one or more giant cells. We have not found this typical CPE

TABLE I. Comparative Reisolation of Respiratory Syncytial Virus from 19 Clinical Specimens.

Specimen no.	Titer[a] in HL cells	Titer[a] in HEp-2 cells
1	1.5	1.0
2	0.5	1.0
3	Neg	Neg
4	0.5	Neg
5	1.0	2.0
6	Neg	Neg
7	2.5	2.5
8	Undilute[b]	Neg
9	Neg	Neg
10	0.5	Undilute
11	1.5	2.5
12	Neg	Neg
13	2.5	2.5
14	0.5	Neg
15	1.5	1.0
16	Neg	Neg
17	1.5	1.5
18	0.5	0.5
19	Neg	Neg

[a] Negative $\log_{10}$ per 0.2 ml.

[b] RS virus isolated in only 1 tube inoculated with original specimen.

TABLE II. Comparison of Titers of Prototype Viruses Propagated in HL and HEp-2 Cell Cultures.

Virus	Passage history	Cell culture in which virus propagated	No. of passages	Cell culture in which virus titrated; titer[a]	
				HL	HEp-2
RS virus	$KB_6L_2HEp\text{-}2_2$	HL	5	(7.2)	6.0
(Long)	HL_1	HEp-2	4	5.5	(6.0)
Adenovirus Type 2	HES_2, $HeLa_{15}$	HL	3	(5.0)	4.0
(Adenoid 6)	KB_{10}	HEp-2	5	4.5	(4.5)
Coxsackie B_5	$MK_7LLCMK_{2\text{-}4}$	HL	3	(7.2)	6.2
(Faulkner)		HEp-2	3	7.5	(6.5)
Herpesvirus hominis	$M_{40}CAM_{32}$	HL	5	(5.5)	4.5
(Armstrong 1166)		HEp-2	5	3.5	(4.5)
Measles	$CE_{13}LLCMK_{2\text{-}10}$	HL	6	(5.0)	4.5
(Edmonston)	$BSC\text{-}1_2$	HEp-2	6	2.5	(2.5)
Mumps	$E_7BSC\text{-}1_7,MA104_{12}$	HL	3	(3.5)	2.5
(Sporn)	$LLCMK_{2\text{-}10}$	HEp-2	3	3.5	(2.5)
Rhinovirus	$MK_2\,KB_{-1}$	HL	3	(4.0)	3.5
Type 1B	$WI38_{-5}$	HEp-2	4	3.0	(5.5)

[a] Titer expressed as $TCID_{50}/0.2$ ml, reciprocal $\log_{10}$.

produced in HL cells by RS virus to be associated with any other respiratory tract virus commonly encountered.

Relative sensitivity of HL and HEp-2 cells for isolation of RS virus from clinical specimens was next determined. Two tubes each of both cultures were simultaneously inoculated with undilute and 10-fold dilutions of specimens from which RS virus had been isolated previously. A total of 19 specimens collected over a 4-yr period were examined and the results are summarized in Table I.

Virus was reisolated in both types of cells from 10 specimens. With 8 the titer detected in one cell system was within 0.5 log of that found in the other. With the other 2 specimens (nos. 5 and 11), a higher titer of virus was detected with HEp-2 than with HL cells, and in both types the CPE appeared rapidly, within the first 5 days of inoculation.

From 3 specimens (nos. 4, 8 and 14), RS virus was recovered only in HL cells. In these cases initial CPE was observed after at least 10 days incubation, and only in those tubes inoculated with undiluted specimen. At this time the HEp-2 tubes were heavily overgrown, and CPE was not recognized. There were no instances in which RS virus was detected in the HEp-2 system and not in HL cells. The virus could not be reisolated from the remaining 6 specimens, which is in keeping with its known lability.

Spectrum of viruses which propagate in HL cells. Suitability of the cell line for propagation of known respiratory viruses was next investigated. Prototype viruses were passed in HL and HEp-2 cells. After a comparable number of passages of a particular virus in either cell system, each was titrated in both HL and HEp-2 cells. Results are shown in Table II; those determined when using the same cell cultures for titration as used for propagation of a virus are given in parentheses. RS virus (Long strain) grew to a very high titer in HL cells, especially when measured in the homologous cell system. HL cultures appeared to be superior to HEp-2 for propagation of certain other viruses; this superiority was most marked for measles. For other viruses there was little difference, but rhinovirus type 1B appeared to adapt more readily to the HEp-2 cells than HL. How-

ever, other heteroploid cells are available which are more suitable for propagation of rhinoviruses than either of the two lines (7).

Discussion. Three cell systems, monkey kidney, WI-38 and a heteroploid line are commonly employed together for the isolation of respiratory viruses (4). The heteroploid line chosen is usually HEp-2 because of its known sensitivity to RS virus (1, 6). HL cells offer certain advantages over HEp-2 for use as the heteroploid line. The growth rate is much slower; therefore, tubes of HL cells can be inoculated with specimens for a longer period after preparation than is possible with HEp-2. The slow growth rate also prolongs the period of effective observation after inoculation of the specimens. The latter fact permits late-developing CPE to be detected, making HL more sensitive for low-titrated clinical specimens of RS virus.

HL cells are also sensitive to other respiratory agents likely to be encountered in clinical specimens, and these viruses also propagate well in the system. Among seven agents tested, only one type of rhinovirus did not develop at least as high a titer in HL as in HEp-2. RS virus itself grew to a very high titer in HL cells, a factor which has been used to advantage in producing antigen for serologic procedures and in adapting the virus to mouse brain *in vivo* experimentation and evaluation of prospective vaccines (2).

Summary. HL, a human heteroploid cell line, was compared to HEp-2 in terms of rate of growth and sensitivity to respiratory viruses. RS virus produced its characteristic cytopathic effect in HL. The cell growth rate was slower than that of HEp-2, permitting recognition of late developing CPE which otherwise would have gone undetected. RS virus propagated to higher titer in HL and the cell line was also sensitive to other respiratory viruses likely to be found in clinical specimens.

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