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CLONES OF MRC-C CELLS MAY BE SUPERIOR TO THE PARENT LINE FOR THE CULTURE OF 229E-LIKE STRAINS OF HUMAN RESPIRATORY CORONAVIRUS

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A clone was selected from the MRC-C continuous heteroploid cell line which was significantly better than MRC-C for the culture of human respiratory coronavirus (HCV) 229E, strain LP. Such clones could prove generally useful for the isolation of HCV-229E from clinical specimens, and for the propagation and assay of laboratory adapted strains.

cloning cell line human respiratory coronavirus

MRC-C is a continuous heteroploid cell line derived from human embryo lung which has been used for the isolation of human respiratory coronavirus 229E strains (HCV-229E) at the Common Cold Unit (Larson et al., 1980). It maintains poorly in roller tube cultures, and often loses its sensitivity to HCV-229E at high passage levels. Heteroploid cell lines may have markedly unstable characteristics (Schnebli et al., 1977) and clones with differing sensitivities to a given virus may be isolated from a single high-passage cell line (see for example Clarke and Spier, 1980). It therefore seemed likely that a clone of MRC-C cells could be selected, which would prove superior to the parent line for the isolation, propagation and assay of HCV-229E.

A number of clones were derived from MRC-C by the culture of single cells in microtitre plate wells, in Ham's F12 medium supplemented with 10% foetal calf serum. There was considerable variation in the gross morphology of the resulting cultures and appearances which varied from fibroblastic (approximately 75% of cultures) to epithelioid, were maintained on subculture.

Clones representative of all morphological types, and MRC-C were compared in parallel experiments for sensitivity to HCV-229E strain LP both by quantal assay of a nasal wash pool taken from volunteers challenge with LP, and plaque assay of a pool of MRC-C adapted LP. The results of these experiments are shown in Table 1. Quantal assay titres ranged from <1.0 to 4.45 $\log_{10}$ $TCID_{50}$/ml and there was considerable variation in how well cells maintained in roller tube culture. However, good maintenance did not appear to be associated with sensitivity to HCV-229E strain

TABLE 1

Comparison of clones by quantal and plaque assay

Clone number	Titre of LP nasal wash pool (log_{10} $TCID_{50}$/ml)	Maintenance in culture	Plaque titre of MRC-C adapted LP (log_{10} PFU/ml)	Plaque morphology and approximate size
1	2.45	Poor	6.05	Pinpoint, clear
2	3.95	Excellent	6.17	Pinpoint, fairly clear
4	3.12	Poor	NT	
5	4.45	Excellent	6.11	Pinpoint, very hazy
6	<1.0	Excellent	5.97	0.5 mm, hazy
8	3.12	Excellent	6.07	Pinpoint, clear
9	3.45	Excellent	6.11	0.5 mm, very hazy
10	3.78	Poor	6.40	1.0 mm, clear
11	3.12	Excellent	6.26	0.75 mm, clear
12	4.12	Fair	5.94	Pinpoint, very hazy
14	2.95	Poor	6.03	0.75 mm, very hazy
15	1.95	Poor	NT	
16	4.28	Excellent	6.23	0.5 mm, clear
17	<1.0	Excellent	5.72	Pinpoint, hazy
20	3.28	Good	6.20	0.75 mm, hazy
21	2.45	Excellent	NT	
MRC-C	1.95	Fair	No plaques	

NT = not tested.

LP (clones 17 and 6 which maintained an exellent appearance in roller tube cultures were amongst the least sensitive). Plaque assay titres ranged from 5.75 to 6.4 log_{10} PFU/ml, and plaque morphologies varied from clear plaques of approximately 1.0 mm in diameter to very hazy, 'pinpoint' plaques. No plaques were produced in MRC-C cells. As might be expected, larger clearer plaques were associated with higher titres. A highly significant positive correlation between the quantal assay and plaque assay titres ($r = 0.643$, $P < 0.01$) supported the idea that sensitivity to HCV-229E strain LP was the dominant variable in both assays. There was no obvious association of cell morphology with any of these properties (data not shown).

Clones also differed in their ability to produce infectious virus. Seven clones and MRC-C were inoculated at a multiplicity of infection of 0.1 with MRC-C adapted HCV-229E strain LP. Infected cultures (5 replicates per clone) were harvested by freezing and thawing when 70% of the cell sheet showed a cytopathic effect, or at 72 h, whichever was the sooner. The yield of infectious virus was expressed as PFU produced per cell. Clones 6, 8, 9, 11, 16 and MRC-C produced 8.6–10.3 PFU/cell, mean 9.25 ± 0.9 (1 SD) PFU/ml, whereas clone 10 produced 16.6 ± 5.7 PFU/cell, and clone 21 2.0 ± 0.9 PFU/cell.

Bacteria or yeasts were not isolated from any of the cultures using standard

techniques, however staining with the DNA binding flourochrome Hoechst strain 33258 (Chen, 1977) showed most cultures to be contaminated with prokaryotic organisms, presumably mycoplasmas. Clones 4, 14 and 15 were heavily contaminated, whilst clones 17 and 20 appeared free of contamination. There was scanty contamination of clone 5, and the remaining clones although infected were not heavily so. The number of mycoplasmas present was not obviously related to sensitivity to HCV-229E strain LP, although those clones which were heavily contaminated maintained poorly in roller-tube cultures.

As a result of these experiments clone 16, which appeared sensitive to HCV-229E strain LP in both the quantal and plaque assays, and maintained well in roller-tube cultures was compared to MRC-C for the detection of HCV-229E strain LP in clinical samples. Forty nasal washes from volunteers challenged with HCV-229E strain LP were inoculated into clone 16 and MRC-C in parallel, in a different random order for each cell type. In clone 16, 28 washes were scored as positive (produced a cytopathic effect) whereas 18 were scored positive in MRC-C ($P < 0.05$, χ^2-test).

Clone 16 has subsequently been used at the Common Cold Unit for the isolation and serotyping by neutralisation of HCV-229E strain LP from over 420 nasal wash samples, and for the assay of neutralising antibody by a microtechnique in over 80 paired serum samples. It has undergone more than 50 subcultures, and although direct comparisons have not been made, it does not seem to have lost its sensitivity to HCV-229E strain LP. Clone 16 continues to maintain well in roller tube cultures, and supports the growth of a further 4 HCV-229E strains which have been tested (data not shown). Such clones should facilitate studies of the natural history and replication in vitro of HCV-229E as they represent a significant advance upon the cell culture systems currently used.

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