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Syncytium production by human coronavirus 229E group viruses

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1. INTRODUCTION

The Coronaviridae are a group of large, RNA-containing enveloped viruses that cause a wide range of diseases in their hosts [1–3]. Many coronaviruses have been readily adapted to growth in cell lines derived from their hosts or other species. The cytopathic effect (cpe) produced by coronaviruses in infected cells is variable [1]. However, the most common feature of the cpe is its focal nature which progresses to involve the entire cell sheet. On the other hand, the cpe formed by some coronaviruses is of a more nonspecific degenerative quality. Most coronaviruses produce a cpe with a tendency to form syncytia, although this cannot at present be considered to be a general feature of the Coronaviridae.

Up to 30 human coronaviruses (HCVs) have been isolated and they all belong to one of two distinct serotypes, named after their prototype viruses 229E [4] and OC43 [5]. In this study, the induction of syncytia in tissue cultures by five closely related 229E group viruses is examined. The characteristics of these syncytia and differences in the syncytia induced by the isolates is reported.

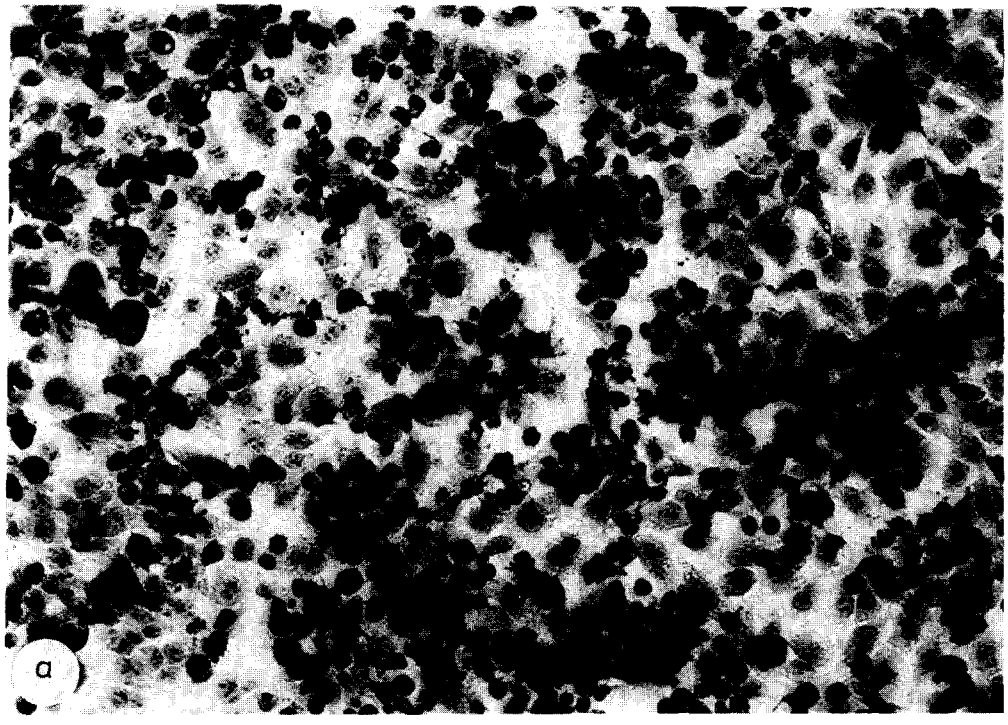
2. MATERIALS AND METHODS

Five HCV 229E viruses, isolates 229E, KI, LP, PR and TO, were used. The 229E prototype strain was originally obtained from Dr. D. Hamre, and KI, LP, PR and TO were isolated in tissue culture at the Common Cold Unit, Salisbury, U.K., from nasal washings of subjects with natural colds [6,7]. The viruses were grown in stationary monolayer cultures of human embryo lung cells of the MRC continuous (MRCc) line, originally obtained from Dr. A.F. Bradburne [8]. The cell monolayers in flat bottles were infected at an input multiplicity of 0.1 infectious particles per cell and, following an adsorption period of 1 h at 33°C, were incubated at 33°C for 24 h in Eagle's BME medium with 2% newborn calf serum. After incubation the cells were harvested, frozen and thawed once, clarified at $2000 \times g$ for 30 min at 4°C, and the supernatant containing infectious virus particles was stored at −70°C until required.

A clone of the MRCcs, called MRC16, was obtained from Dr. R. Phillpotts and used together with MRCcs, for syncytial formation studies. The five HCV 299E-group viruses grew to similar titres in both MRCcs and MRC16s. Cells in roller-culture tubes were infected as described above and the monolayers were examined for cpe at regular intervals up to 7 days after infection.

3. RESULTS

The cpe produced in rolled monolayers of MRCc and MRC16 cells by the HCV 229E group isolates 229E, KI, LP, PR and TO was examined under different cultural conditions. Two types of cpe were observed in HCV-infected cells. One type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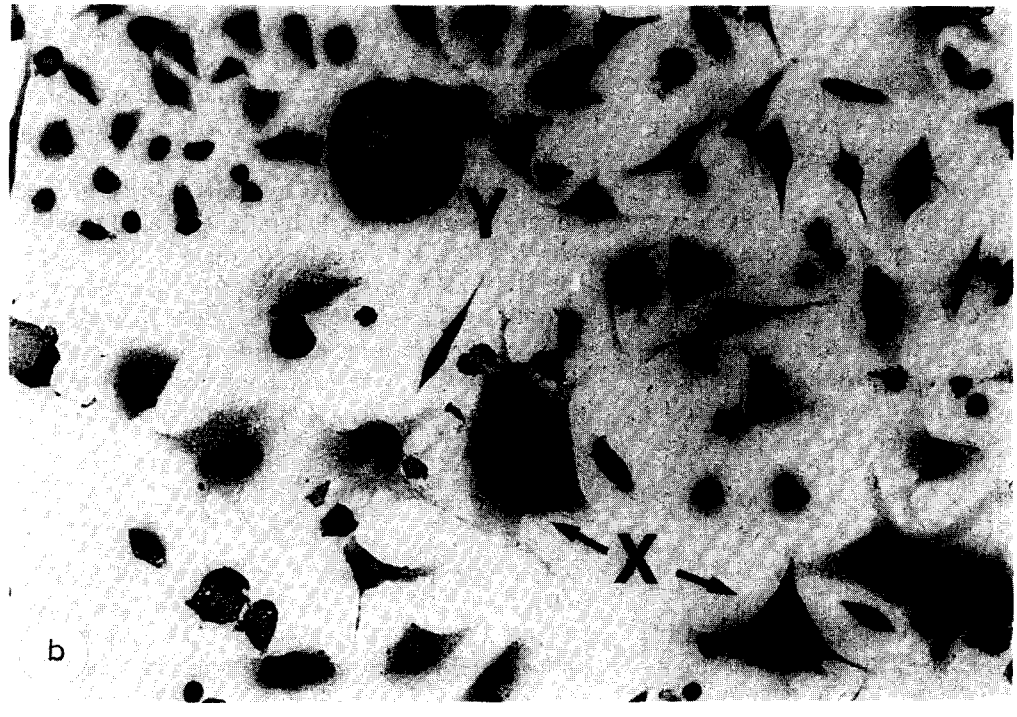
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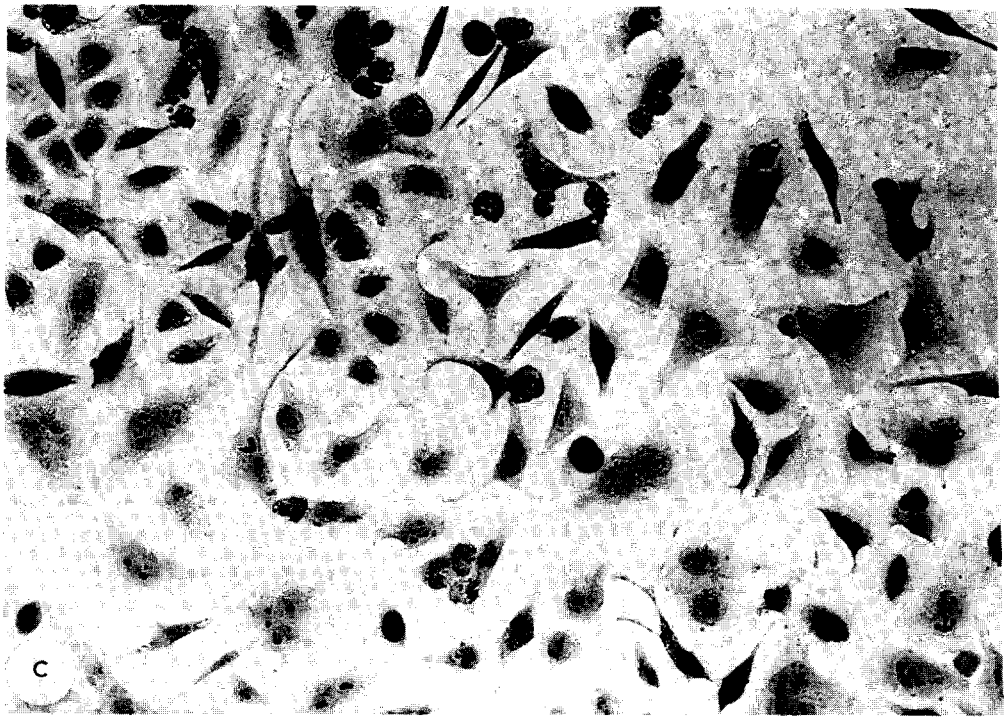

Fig. 1. Monolayers of MRC16 cells stained with haematoxylin and eosin. (a) Passage number 12 cells 4 days after inoculation with HCV KI infected at an moi of 0.01 and showing diffuse rounding up of cells. (b) Passage number 13 cells 6 days after inoculation with HCV KI infected at an moi of 0.001 and showing syncytia. Two types of syncytia were seen: cells with nuclei around the periphery (X) and large vacuolated cells (Y). (c) Passage number 13 uninfected cells. Magnification × 150.

was a diffuse rounding-up of cells over the whole of the cell sheet (Fig. 1a). The other cpe consisted of a diffuse rounding-up of cells together with the formation of syncytia (Fig. 1b). The multinucleate cells first appeared as clear refractile cells which developed a granular appearance. In later stages giant cells with nuclei around the periphery, and large vacuolated cells, sometimes with no nuclei, were observed. Both types of cpe were observed by 4 days after infection, and the cpe gradually progressed to the whole monolayer. An uninfected cell monolayer is shown in Fig. 1c. Syncytia were only seen in infected rolled monolayers: infected stationary monolayers produced only diffuse rounding-up of cells.

The cpe observed in all infected MRCc cells and high passage (greater than 20 passes) MRC16 cells was always of the non-syncytial type. However, both types of cpe were obtained in infected MRC16 cells of less than 20 passes. The proportion of syncytia in these low passage MRC16s varied with a number of factors including the passage number, the medium used, the multiplicity

Table 1

Cpe produced in MRC16 cell monolayers on infection with HCV 229E group isolates

HCV isolate [a]	Proportion of cpe in form of syncytia [b]
229E	+ + +
KI	+ +
LP	+ +
PR	+ +
TO	+

[a] All isolates were inoculated at 0.01 infectious particles per cell on to MRC16 monolayers at pass 12 in rolled tubes.

[b] Cpe recorded at 4 days post inoculation. + + +, 40–60% of cpe in form of syncytia; + +, 20–40%; +, 0–20%.

of infection (m.o.i.) and the HCV isolate used. Table 1 shows the results of a typical experiment. Different HCV isolates produced different proportions of cpe, with up to 60% of the cpe in the form of syncytia for 229E, but less than 20% for TO. Infection with low m.o.i. (0.01 or less) with HCV isolates produced higher proportions of syncytia in the cpe than higher m.o.i.

4. DISCUSSION

The cells and cultural conditions used are crucial in obtaining growth of HCVs in tissue culture [9]. Although most 229E group HCVs grow in tissue culture [9–12], the only tissue-culture adapted OC43 group viruses are OC38 and OC43 [12–14] and these are probably identical [1]. All these reports, with one exception [13], describe the cpe for HCVs as a diffuse rounding-up of cells around the edges of the monolayers, although in some cases it is more focal. However, Bruckova et al. [13] observed that the cpe produced in rhesus or vervet monkey kidney cells and in BSC1s was focal with a tendency to the formation of syncytia which progressed to involve the entire cell sheet. Virus-induced cell fusion of human macrophages by HCV 229E has also been observed [15], although the proportion of multi-nucleate cells in the infected macrophage population was small compared with that found in studies with the coronavirus, mouse hepatitis virus strain 3 [16,17].

In this report we have shown that several different HCV isolates can induce syncytia, although the type and age of the cells and their cultural conditions were crucial for the formation of virus-induced syncytia. This may explain why this phenomenon has been so difficult to observe in the past. Furthermore, our results show that under defined conditions some antigenically closely related HCV isolates can be differentiated according to the ability to induce syncytia. Thus, it appears that the induction of syncytia, which has been frequently observed with other coronaviruses [1–3], is also common to HCVs, and this feature may be characteristic of all coronaviruses.

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