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Differentiation of smallpox and camelpox viruses in cultures of human and monkey cells

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SUMMARY

The cytopathic effect of smallpox and camelpox viruses has been compared in cell cultures derived from human and monkey tissues. The two viruses could easily be distinguished in HeLa, GMK–AH1, BSC–1 and WISH cells, camelpox producing multinucleate giant cells and smallpox producing rounding up of individual cells. In other cells (LLC–MK2, HEK and 2RhK) the differences were not so marked, and in some (VERO, HEL, K and HuTh) no differences were seen.

INTRODUCTION

Recent work in this laboratory has shown that the techniques commonly used in smallpox diagnostic laboratories fail to distinguish between strains of camelpox virus isolated in Iran, and strains of smallpox virus isolated in Tanzania (Baxby, 1972). However, subsequent preliminary experiments (Bedson, 1972) suggest that camelpox and smallpox viruses produce different types of cytopathic effect (CPE) in HeLa cells. Previous studies had failed to differentiate the viruses in camel (Mirchamsy & Ahourai, 1971), rabbit and chick cells (Baxby, 1972). The present paper extends these observations by comparing the CPE produced by camelpox and smallpox viruses in a number of cell lines and cell strains of human and monkey origin.

MATERIALS AND METHODS

Virus strains

Three strains of camelpox virus, CM–G1, CM–G2 and CM–S, whose origins have been described elsewhere were used (Baxby, 1972). Also used were the International Reference Strain of smallpox virus (Harvey), two strains of smallpox virus, EA8 and EA17, isolated in Tanzania (Bedson, Dumbell & Thomas, 1963), and strain 64–7275, a so-called 'white' poxvirus isolated from the tissues of a healthy monkey and so far indistinguishable from smallpox virus (Gispen & Brand-Saathof, 1972; Marennikova, Selukhina, Maltseva & Ladnyi, 1972).

Cell cultures

Cell cultures were grown in $6 \times \frac{1}{2}$ in. tubes in one of four media: Eagle's MEM with (i) adult or (ii) foetal calf serum, or Parker's 199 with (iii) adult or (iv) foetal calf serum.

The monkey cells used (with medium shown in parentheses) were as follows: secondary rhesus kidney, 2RhK (iii), three lines of transformed green monkey kidney cells, BSC–1 (ii), GMK–AH1 (ii), VERO (iv) and one line of transformed rhesus monkey kidney cells LLC–MK2 (iii).

The human cells used were two lines of transformed amnion cells, K (iii) and WISH (iv), HeLa cells (i), cell strains derived from human embryo lung, HEL (ii), and kidney, HEK (ii), and secondary human thyroid cells HuTh (iii).

Confluent cell sheets were infected with various doses of virus calculated to give all stages of CPE from very fast total destruction of cell sheets, to the much slower development of isolated foci of infection. Cell sheets were fixed in 5% formal-saline and were photographed unstained using phase-contrast optics (McCarthy, 1960).

RESULTS

The results obtained are summarized in Table 1. The CPE produced by the strains of smallpox virus and the 'white' poxvirus were the same. No differences were found in the type of CPE produced by the three strains of camelpox virus.

The results could be divided into three categories. Cells in which clear-cut differences were found between the behaviour of camelpox and smallpox viruses, those in which differences were not so clear-cut and those in which no differences were found. Any differences found were consistent whatever the dose of virus used. With high doses (10^5 to 10^6 pock-forming units/ml.) CPE was detectable within 24 hr. and soon spread to involve the whole cell sheet. With lower doses (10^2 to 10^3 pfu/ml.) CPE was not detected until the second or third day and resulted in localized plaque production.

Clear differences were found between smallpox and camelpox viruses in HeLa (Plate 1, figs. 1, 2), GMK–AH1 (figs. 3, 4), BSC–1 and WISH cells. In all these cells camelpox virus produced multinucleate giant cells (GC CPE), whereas the CPE of smallpox virus was characterized by the rounding up of infected cells (round cell (RC) CPE), with the occasional production of cells with long cytoplasmic strands (strand cell (SC) CPE). With low doses smallpox virus produced characteristic 'hyperplastic foci' – small aggregations of heaped-up cells (Pirsch & Purlson, 1962). Camelpox virus produced small holes in the cell sheet caused by the detachment of multinucleate cells.

In LLC–MK2 (figs. 5, 6), HEK and 2RhK cells the differences were not so marked. In LLC–MK2 cells smallpox virus tended to produce round cell CPE with an occasional strand cell (fig. 5), whereas camelpox virus produced a mixed strand cell/round cell CPE (fig. 6). In 2RhK cells smallpox virus produced a mixed round cell/giant cell CPE and camelpox virus a mixed strand cell/giant cell CPE. In HEK cells smallpox virus produced strand cell CPE whereas camelpox virus in addition produced occasional giant cells.

Table 1. *Cytopathic effects of smallpox and camelpox viruses in human and monkey cells*

Cell type	Designation	Viruses	
		Smallpox	Camelpox
Transformed green monkey kidney	VERO	SC–RC	SC–RC
Transformed green monkey kidney	BSC–1	RC–SC	GC
Transformed green monkey kidney	GMK–AH1	RC(SC)	GC
Transformed rhesus monkey kidney	LLC–MK2	RC(SC)	SC–RC
Secondary rhesus monkey kidney	2RhK	RC–GC	SC–GC
Human cervical carcinoma	HeLa	RC	GC
Transformed human amnion	K	RC	RC
Transformed human amnion	WISH	RC	GC
Diploid human embryo lung	HEL	SC(GC)	SC(GC)
Diploid human embryo kidney	HEK	SC	SC(GC)
Secondary human thyroid	HuTh	SC	SC

SC = strand cell CPE, GC = giant cell CPE, RC = round cell CPE. Bracketed entries refer to occasional or very minor type of CPE. For example, SC–RC = predominantly SC with some RC. RC–SC = predominantly RC with some SC. SC(GC) = predominantly SC with occasional or very minor GC.

In HEL (figs. 7, 8), VERO, K and HuTh cells no differences were detected in the type of CPE produced by the viruses.

DISCUSSION

The results described here, although failing to distinguish between smallpox and the 'white' poxviruses, confirm and extend those of Bedson (1972) in indicating that the viruses of smallpox and camelpox are not identical. At the same time the results provide easy means of distinguishing them. It should be remembered that consistent results have not always been obtained with smallpox virus in cell cultures (Mika & Pirsch, 1960; Bedson & Dumbell, 1964; Netter & Piat, 1969; Gispen & Brand-Saathof, 1972) and that a giant cell producing a variant of smallpox virus has recently been selected by repeated passage through monkey kidney cells (Tsuchiya & Tagaya, 1972). Nevertheless, consistent results have been obtained in the author's laboratory with both smallpox and camelpox material passaged through various animals (mouse, monkey, chick embryo) or adapted to various cell cultures (VERO, HeLa, BSC–1).

The results of these and earlier studies (Baxby, 1972) suggest that the ease with which smallpox and camelpox viruses can be distinguished upon isolation depends essentially on the choice of cell used to detect CPE. Hence it is hoped that the information presented here, that the viruses can be differentiated in a number of cell cultures, may be of value to those interested in the comparisons between smallpox and related viruses.

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REFERENCES

BAXBY, D. (1972). Smallpox-like viruses from camels in Iran. *Lancet* ii, 1063.

BEDSON, H. S. (1972). Camelpox and smallpox. *Lancet* ii, 1253.

BEDSON, H. S. & DUMBELL, K. R. (1964). Hybrids derived from the viruses of variola major and cowpox. *Journal of Hygiene* **62**, 147.

BEDSON, H. S., DUMBELL, K. R. & THOMAS, W. R. G. (1963). Variola in Tanganyika. *Lancet* ii, 1085.

GISPEN, R. & BRAND-SAATHOF, B. (1972). 'White' poxviruses from monkeys. *Bulletin of the World Health Organization* **46**, 585.

MCCARTHY, K. (1960). An adaptor for examining tissue cultures in test tubes by phase contrast microscopy. *Virology* **12**, 313.

MARENNIKOVA, S. S., SELUKHINA, E. E., MALTSEVA, N. N. & LADNYI, I. D. (1972). Poxviruses from clinically ill and asymptomatically infected monkeys and a chimpanzee. *Bulletin of the World Health Organization* **46**, 613.

MIKA, L. & PIRSCH, J. (1960). Differentiation of variola virus from other members of the poxvirus group by the plaque technique. *Journal of Bacteriology* **80**, 861.

MIRCHAMSY, H. & AHOURAI, P. (1971). Comparative adaptation of some poxviruses in two cell systems. *Archives of the Institute Razi, Teheran* **23**, 93.

NETTER, R. & PIAT, A. (1969). Essais de culture de quatre souches de virus varioliques sur cellules RK13 et BHK21. *Annales de l'Institut Pasteur* **116**, 820.

PIRSCH, J. & PURLSON, E. H. (1962). A tissue culture assay for variola virus based on the enumeration of hyperplastic foci. *Journal of Immunology* **83**, 632.

TSUCHIYA, Y. & TAGAYA, I. (1972). Isolation of a multinucleated giant-cell-forming and haemagglutinin negative variant of variola virus. *Archiv für die gesamte Virusforschung* **39**, 292.

EXPLANATION OF PLATE

Cytopathic effects produced by smallpox and camelpox viruses. Unstained, phase contrast, × 325.

Fig. 1. Smallpox virus in HeLa cells.

Fig. 2. Camelpox virus in HeLa cells.

Fig. 3. Smallpox virus in GMK–AH1 cells.

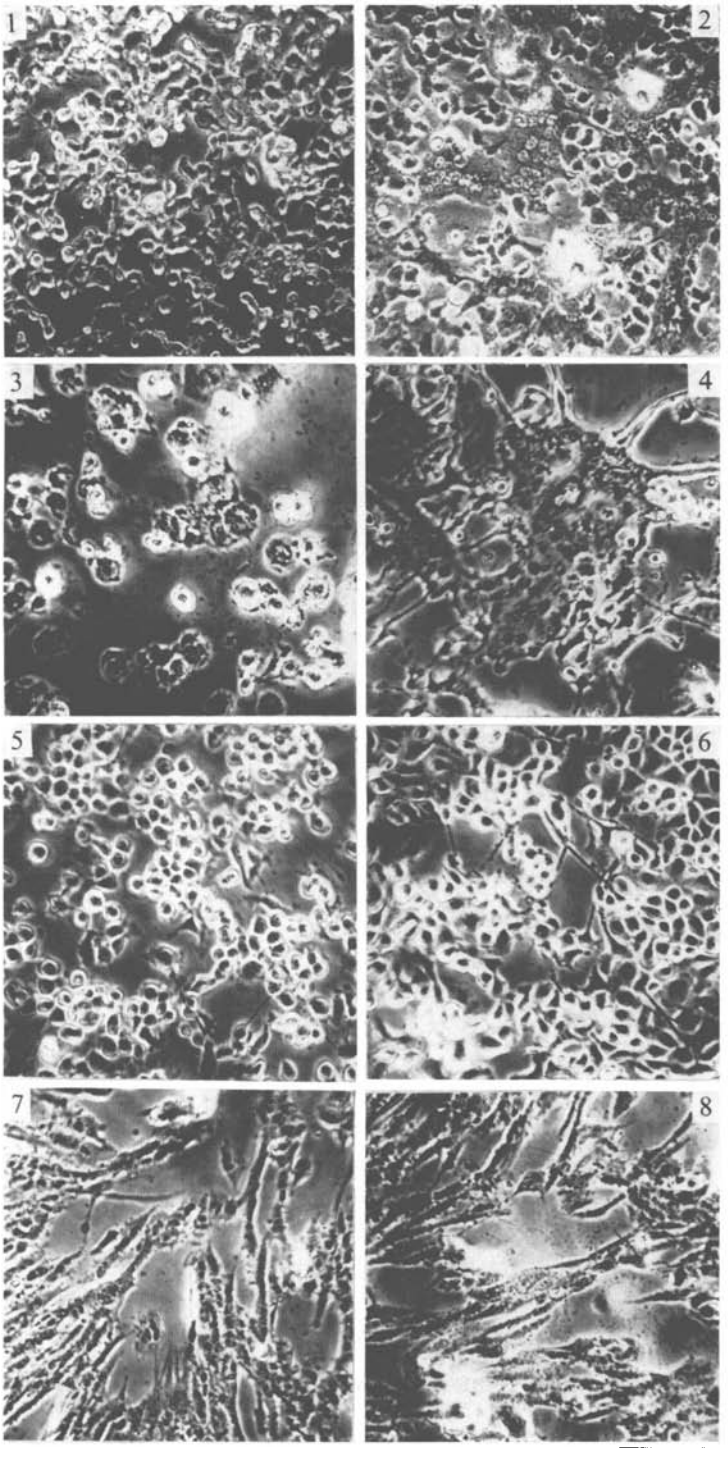
Fig. 4. Camelpox virus in GMK–AH1 cells.

Fig. 5. Smallpox virus in LLC–MK2 cells.

Fig. 6. Camelpox virus in LLC–MK2 cells.

Fig. 7. Smallpox virus in HEL cells.

Fig. 8. Camelpox virus in HEL cells.



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