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AUTOCOMPLEMENTATION OF INFLUENZA VIRUS DEFECTIVE INTERFERING PARTICLES: CELLS AT HIGH MULTIPLICITY INFECTED WITH DEFECTIVE INTERFERING PARTICLES PRODUCE DEFECTIVE VIRIONS

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Summary. — MDCK cells were infected with incomplete influenza virus alone, with standard influenza virus alone, or simultaneously with both preparations at a wide input range. The analysis of one-cycle yields suggests that a cell infected at high multiplicity with defective interfering (DI) particles only is able to produce a substantial yield of non-infectious virions even in the absence of concomitant infection with the infectious virus.

Key words: *DI particles*; *influenza virus*; *genome expression*

The genome of defective interfering (DI) influenza virus particles has been extensively studied in recent years (Bean and Simpson, 1976; Crumpton *et al.*, 1978; Nayak *et al.*, 1978; Janda *et al.*, 1979; Davis and Nayak, 1979; Davis *et al.*, 1980; Pons, 1980; Ueda *et al.*, 1980). The majority of authors agree that the defectiveness of influenza DI particles may be understood assuming that at least one of genes Pl-P3 is substituted by a defective segment (Crumpton *et al.*, 1978; Davis and Nayak, 1979; Davis *et al.*, 1979; Janda *et al.*, 1979; Nakajima *et al.*, 1979). It seems that while in some influenza DI populations the same gene is lacking in all particles (Nakajima *et al.*, 1979; Pons, 1980), other populations may be heterogeneous in this respect, so that different gene (anyone of P1-P3) may be lacking in different DI particles (Nayak *et al.*, 1978; Davis and Nayak, 1979; Janda *et al.*, 1979; Ueda *et al.*, 1980). Such a population would be expected to exert some kind of autocomplementation, since in a cell infected with two DI particles lacking different genes the whole set of RNA segments would be present. In our previous studies (Kaverin *et al.*, 1980) we have presented data suggesting that multiple infection with influenza DI particles leads to the synthesis of virus-specific proteins and to a conversion of the infected cell into a haemadsorbing one by 8 hr post infection (that is, after a one-cycle reproduction). However, we could not register formation of infectious centres in cells multiply infected with DI particles.

In this paper we present results suggesting that MDCK cells infected with influenza DI particles at high multiplicity of infection (m.o.i.) produce

Table 1. Virus yields in MDCK cultures infected with incomplete and standard influenza virus

Virus strain	Final dilution in the inoculum: Incomplete virus	Final dilution in the inoculum: Standard virus	One-cycle yield: HAU	One-cycle yield: PFU/culture
A/WSN/33	10^{-1}	10^{-2}	32	2.2×10^3
	10^{-1}	10^{-3}	16	1.0×10^2
	10^{-1}	10^{-4}	16	1.1×10^2
	—	10^{-2}	32	2.1×10^5
	—	10^{-3}	2	3.6×10^4
	—	10^{-4}	2	4.0×10^3
	10^{-1}	—	16	1.2×10^2
	10^{-2}	—	2	4.3×10^2
A/FPV/Weybridge	10^{-1}	10^{-2}	32	3.6×10^4
	10^{-1}	10^{-3}	32	ND
	10^{-1}	10^{-4}	32	7.1×10^2
	—	10^{-2}	32	9×10^6
	—	10^{-3}	4	ND
	—	10^{-4}	2	1.1×10^4
	10^{-1}	—	32	8.0×10^2

A/WSN/33: standard virus = 1024 HAU, 1.4×10^7 PFU/ml;
incomplete virus = 256 HAU, 1.4×10^4 PFU/ml;
A/FPV/Weybridge: standard virus = 1024 HAU, 2.6×10^8 PFU/ml;
incomplete virus = 256 HAU, 2.5×10^5 PFU/ml;
Culture fluid was harvested 10 hr post infection.

a substantial yield of defective (non-infectious) virions. This production does not need a concomitant infection of the cell with a DI particle and an infectious virion.

MDCK cells were obtained from the tissue culture laboratory of the D. I. Ivanovsky Institute of Virology. The procedures for cell cultivation and infection as well as for preparation of incomplete virus A/WSN/33 and A/FPV/NWeybridge strains by undiluted passages in chick embryos have been described (Kaverin *et al.*, 1980). Plaque titration was performed in chick embryo cell (CEC) culture utilizing trypsin-containing agar overlay (Appleyard and Maber, 1974). Trypsin (15 μg/ml) was present also in the inoculum during the adsorption period. The sensitivity of the plaque titration was by 1.0—1.5 $\log_{10}$ lower than the sensitivity of the end-point titration in chick embryos. To label the A/WSN/33 virus, CEC were infected at m.o.i. 5—10 PFU/cell; at 2 hr post infection the maintenance medium (Eagle's MEM) was replaced with the labelling medium (MEM) diluted 10 times with Hanks' BSS containing 0.2% bovine serum albumin and 925 kBq/ml of ^{14}C-chlorella hydrolysate. The culture fluid was collected at 10 hr post infection, and the virus was purified as described earlier (Sklyanskaya *et al.*, 1980). For analysis of the yields, MDCK cells were infected with standard and incomplete influenza virus at appropriate dilutions and incubated for 10 hr at 37°C. The culture fluid (double MEM with 0.2% bovine serum albumin) was collected and clarified by low speed centrifugation: HA and infectivity titres were determined.

Von Magnus virus preparations used in these studies (the 2nd undiluted chick embryo passage) contained ≈ 1000 times lower concentration of infectious virus and 8 times lower haemagglutination titre than the standard virus preparations (see Table 1). MDCK cells infected with a low dilution (1 : 10) of incomplete virus produced a substantial yield of haemaggluti-

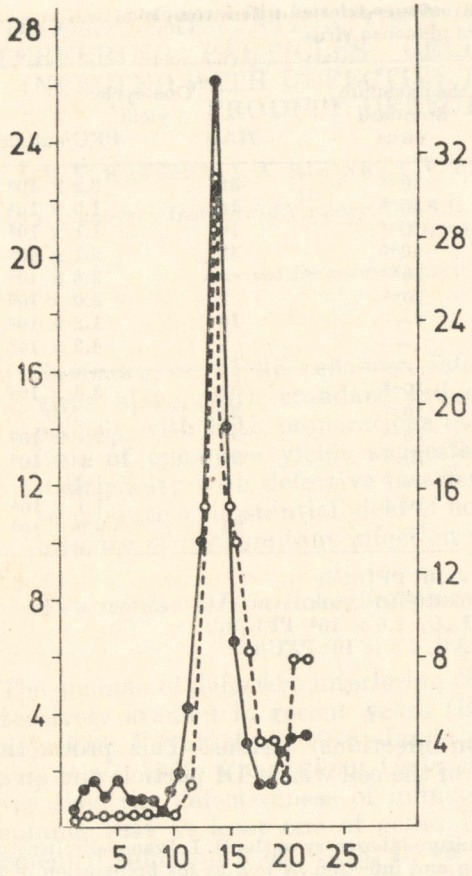

Fig. 1.

Sedimentation analysis of the virus produced in MDCK cells infected with the incomplete influenza A/WSN/33 virus MDCK cells were infected with incomplete virus diluted 1 : 10. The culture fluid harvested 10 hr post infection was layered on sucrose gradient (15—40%) and centrifuged in 3 × 25 bucket rotor of Super-speed 50 ultracentrifuge for 50 min at 4 °C and 24,000 rev/min. The gradient were fractionated and haemagglutinin titre was determined in the fractions (○ — — ○). ^{14}C-labelled purified A/WSN/33 virus was centrifuged in a separate gradient (● —— ●).

Abscissa: fraction number; left ordinate: cpm × 10^{-3}; right ordinate: HAU/ml.

nating material, equal to (or slightly lower than) the yield produced by the cells infected with the standard virus at high m.o.i. The sedimentation characteristics of the haemagglutinating material were identical to those of the purified labelled virus (Fig. 1). Thus the material seemed to contain influenza virions; however, its infectivity was extremely low (Table 1). The cells infected with higher dilutions of incomplete virus produced no detectable haemagglutinin, although such dilutions did exert an interfering effect against superinfection with standard virus (Table 2) reducing of the PFU/HAU ratio in the progeny. The reduction of PFU/HAU ratio indicates that all cells (or their vast majority) were infected with DI particles. This suggests that the infection with a single DI particle is not sufficient for the production of virus particles. Since such production did occur in cells infected with low dilutions of incomplete virus, we had to discern between the two possibilities. The virion formation might be a result of the autocom-

Table 2. Interference ability of A/FPV/Weybridge incomplete virus in MDCK cells

Final dilution in the inculum		One-cycle yield	
Incomplete virus	Standard virus	HAU	PFU/culture
10^{-1}	10^{-1}	64	3.2×10^5
10^{-2}	10^{-1}	64	8.7×10^5
10^{-3}	10^{-1}	64	3.9×10^6
—	10^{-1}	128	7.7×10^6

plementation of DI particles in cells infected at high m.o.i. or it might be due to a concomitant infection of the cells with DI particles and infectious particles, since a small amount of the latter is present in the incomplete virus preparations.

In order to discriminate between these assumptions, we performed a simultaneous infection of the cells at high m.o.i. with incomplete virus and serial dilutions of standard virus (Table 1). A parallel set of cultures was infected with serial dilutions of the standard virus alone. The measurement of haemagglutinin concentration in the yields indicates that the amount of standard virus equal to the infectious virus content in the incomplete virus is too small to induce the accumulation of any detectable amount of haemagglutinin in the culture fluid. This seems to be a strong indication in favour of the view that the accumulation cannot be ascribed to the presence of infectious particles in Von Magnus virus, and should be regarded as a function of DI particles. One might suggest as alternative explanation that the cell infected with a DI particle and an infectious virion would produce a much higher yield of virions, than the cell infected with the infectious virus alone. This, however, seems not to be the case, since cells infected with a sufficiently high dose of standard virus alone produce a yield of virions which is not lower than that produced by the cells infected with the same dose of standard virus together with the incomplete virus (Table 1).

The data presented in this paper strongly suggest that the cells infected with several DI influenza particles are able to produce virions. These virions are as expected mostly noninfectious, since a cell infected with several DI particles does not only contain the whole set of RNA segments, but also the DI genome segments, ready to replicate and replace the genome segments in the progeny virus. A close inspection of the data in Tables 1 and 2 reveals that an increase of standard virus concentration in the mixed inoculum results in an increase in the infectious titre of the yield: this illustrates a competitive nature of the interference exerted by DI segments.

In conclusion, the results presented in this paper seem to corroborate the concept of Von Magnus that influenza virus is a heterogeneous population, where different virions are lacking different viral genes so that some kind of functional complementation may occur at a high m.o.i. The data also explain the absence of multiplicity reactivation sensu stricto (that is, formation of infectious centres by the cells multiply infected with DI influenza particles).

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