Complete and Abortive Infection of Cell Cultures by Influenza A2 Virus

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SUMMARY

The reproduction of a mutant of A2/SINGAPORE/57, selected after repeated passages in monkey kidney cells, and the synthesis of its subviral components were followed in two systems: in monkey kidney cells, in which new infectious virus is formed, and in human diploid cells, in which the growth cycle of this virus is abortive. The formation of subviral antigens was investigated by both immunofluorescence and complement-fixation tests. No marked differences were found between the kinetics of synthesis of the V and S antigens and their total yields in both systems. Migration of the S antigen from the nuclei was observed in A2/SINGAPORE virus infected by human diploid cells.

INTRODUCTION

Influenza viruses have been reported to replicate in a variety of tissue cultures (Wong & Kilbourne, 1961; Choppin, 1962; Lehman-Grube, 1963; Grossberg, 1964; Suguiru & Kilbourne, 1965). Depending on the origin of the cells and on the virus, the growth cycle may be either complete, with formation of infectious progeny, or abortive. If abortive, subviral components are synthetized but not new infectious virus. Several types of abortiveness have been described and several mechanisms suggested as explanations (Henle, Girardi & Henle, 1955; Deinhardt & Henle, 1957; Fraser, 1967; Hillis, Moffat & Holtermann, 1960).

In this paper results are presented of experiments with a variant derived from A2/SINGAPORE/57 virus after repeated propagations in monkey kidney cells. The virus was grown in monkey kidney cells, in which it can be propagated serially, and in a human diploid cell strain, in which the infection is abortive. The development of subviral components of the influenza virus was investigated by both immunofluorescence and complement-fixation.

METHODS

Virus. A variant of influenza A2/SINGAPORE/57 virus denoted A2/SINGAPORE MK-15 was used; it was selected after 15 passages in rhesus monkey kidney cells (Vonka, 1965). This virus produces clear cytopathic changes in these cells. For the experiments to be described, the first chick embryo passage of the MK-15 virus was used.

Sera. Specific antisera to the S and V antigens of A2/SINGAPORE MK-15 virus were prepared in guinea pigs (Závadová, Kutinová & Vonka, 1967).

Cells. Primary rhesus monkey kidney cells were cultivated as described previously.
(Vonka, 1965). Human diploid cells strain LEP-14 were derived from the lungs of a 3-month human embryo. At the passage levels used, the number of cells exhibiting alterations of karyotype never exceeded 10%. Their cultivation was the same as described previously (Kutinová, Vonka & Řezáčová, 1967). Infected cultures were maintained in EPL medium containing 0.3% lactalbumin hydrolysate and a mixture of growth active proteins of calf serum (Michl, 1961).

Growth-cycle experiments. The reproduction of the A2/SINGAPORE/57 MK-I5 virus was studied in parallel in monkey kidney cells and human diploid cells. The cells were grown in Petri dishes seeded with $2 \times 10^6$ cells. The multiplicity of infection was the same, i.e. about 10 EID$_{50}$/cell in both systems. Two ml. of the virus suspension diluted in maintenance medium were added to each of the cultures. After 1 hr incubation at $37^\circ$ the unadsorbed virus was rinsed off and 5 ml. of maintenance medium was added. The same maintenance medium was used for both types of culture. Infected cultures were placed in a CO$_2$ incubator at $37^\circ$. At intervals, two cultures of each type were taken out. Their fluids were drawn off and were cleared of the floating cells by low-speed centrifugation. The cell sheets were washed with phosphate buffered saline + 0.02% EDTA and the cell suspension was resuspended in the same volume of maintenance medium, after having first been washed in PBS. This final cell suspension was frozen and thawed three times. Both fluid-phase and cell-phase preparations were kept at $4^\circ$ until investigation. The infectivity tests and complement-fixation tests on the materials from each experiment were performed simultaneously using the same reagents.

In parallel, Petri dishes containing cover glasses were seeded with cells and infected with virus, as described above. At 1 hr intervals the cover-glass cultures were picked up for immunofluorescence tests. All cover-glass cultures from each experiment were tested simultaneously.

Immunofluorescence test. The indirect technique described previously was used (Závadová et al. 1967).

Complement-fixation reaction. The drop technique on plexiglass panels was employed as described previously (Závadová et al. 1967). The volume of each reagent was 0.03 ml. The virus fluids diluted 1/1 to 1/32 were tested for the presence of either V or S complement-fixing antigens in the presence of 1.7 units of complement, 10 units of haemolysin and excess of the respective antibody. The final concentration of sheep red blood cells was 0.06%. Inhibition of at least 50% haemolysis was considered a positive reaction.

Haemagglutination test. A ½ ml. of virus suspension was mixed with 0.5% chick red blood cells. The mixture was incubated for 60 min. at room temperature. Agglutination of more than 75% of cells was considered a positive result.

Virus titrations. Tenfold dilutions of virus in precooled PBS were inoculated into rhesus monkey-kidney tube cultures. Five tubes were used for each virus dilution. The cultures were inspected for cytopathic changes on the 6th day.

RESULTS

The formation of subviral components of influenza virus was tested simultaneously in monkey kidney cells and human diploid cells in three consecutive experiments which all gave similar results (Fig. 1, 2).
Complete and abortive growth of influenza virus

In one step growth experiments with A2/Singapore virus in monkey kidney cells, new virus appeared in the cell phase 5 hr after infection and extracellular virus appeared about 2 hr later. Greatest titres within the cells were found between the 10th and 13th hr after infection, in the fluid phase after the 17th hr. The appearance of haemagglutinin in both phases followed closely upon the appearance of the new infectious virus. The V and S antigens were detected by complement-fixation test in the cell phase 10 and 8 hr after infection, respectively. In the fluid phase, the V antigen was detected by complement-fixation test simultaneously with the appearance of haemagglutinin; however, the appearance of the S antigen was significantly delayed. It was first detected in the 24 hr sample, when the first cytopathic changes were already visible (Fig. 1).

In human diploid cells, in which very little or no new infectious virus was produced, the amounts of both V and S antigens formed were at least as great as in monkey kidney cells. Also the kinetics of antigen production were comparable in both sorts of cell (Fig. 2).

Immunofluorescence tests were made with both sorts of cell in parallel (Table 1). In both, the S antigen was first detected in the nuclei 3 hr after infection (Pl. 1, fig. 1, 2). The V antigen was observed in the perinuclear zone 2 hr later. No marked differences in the intensity of fluorescence between monkey kidney cells and human diploid cells were observed. Beginning with the 5th hr in monkey kidney cells and the 6th hr in

Fig. 1. The growth of A2/Singapore virus MK-15 in monkey kidney cells. •—•, Infective virus; ○—○, haemagglutinin; ■—■, S antigen; □—□, V antigen.
human diploid cells, the S antigen migrated from the nucleus into the cytoplasm (Pl. 1, fig. 3, 4). After 24 hr most of the S antigen in both sorts of cell was detected outside the nucleus (Pl. 1, fig. 5, 6).

![Graph showing cell phase and fluid phase](image)

**Fig. 2.** The growth of A2/SINGAPORE virus MK-15 in human diploid cells. ●, Infective virus; ○, haemagglutinin; ■, S antigen; □, V antigen.

**Table 1.** Detection by immunofluorescence of S and V antigens in rhesus monkey kidney cells and human diploid cells infected with A2/SINGAPORE MK-15 virus

<table>
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<tr>
<th>Time (hr)</th>
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<td>N</td>
<td>C</td>
<td>V antigen</td>
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N, nucleus; C, cytoplasm; -, no fluorescence; +, specific fluorescence; *, most of the S antigen outside nucleus.
**Complete and abortive growth of influenza virus**

**DISCUSSION**

The appearance of the S antigen in the fluid phase of the monkey-kidney cultures coincided with the development of the first clear cytopathic changes. It is therefore probable that no significant release of free S antigen from intact cells occurred, as was also observed by Low, Eaton & Uretsky (1962), who studied the replication of influenza virus in ascites tumour cells. Like Hillis et al. (1960) and Breitenfeld & Schäfer (1957), we detected the V antigen by immunofluorescence only in the cytoplasm of infected cells, and it appeared there after the S component had been detected in the nuclei. This is at variance with some results obtained with labelled amino acids (Scholtissek et al. 1964), which indicated that both substances are synthesized in the nucleus and that their synthesis is initiated at the same time.

In the human diploid cells little or no new infectious A2/SINGAPORE virus was formed although the production of subviral components was approximately as effective as in monkey kidney cells, in which the infectious cycle was complete. Like Fraser (1967), who studied the abortive cycle of the ME1 strain of influenza virus in BHK 21 cells, we too noted the migration of S antigen from the nucleus into the cytoplasm. Thus the abortive growth of influenza A2/SINGAPORE in LEP cells differs from those observed with other myxoviruses in other cells, in which no migration of S antigen from the nuclei was detected (Franklin & Breitenfeld, 1959; Hillis et al. 1960; Pristošová, Leššo & Szanto, 1966). Fraser's findings and ours indicate that the cessation of S antigen migration is not the only mechanism which might be responsible for abortive influenza virus cycles.

Another observation relating to the distribution of the S antigen within the culture may be of interest. The complement-fixing S antigen appeared earlier in the fluid phase in human diploid cells than in monkey kidney cells. This was observed in all three experiments. The interval varied from 7 to 28 hr. It remains to be determined whether this difference was directly associated with the abortiveness of the cycle in human diploid cells, i.e. with the failure to assemble a significant quantity of the S antigen with the other virus components in the infectious virions, or whether it was caused by a different capacity of the two cell types for releasing the virus subunits.

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**REFERENCES**


H. ZÁVADOVÁ AND OTHERS


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EXPLANATION OF PLATE

Fig. 1. Nuclear staining in monkey kidney cells 4 hr after infection.

Fig. 2. Nuclear staining in human diploid cells 4 hr after infection.

Fig. 3. Migration of S antigen in monkey kidney cells from nucleus to cytoplasm 5 hr after infection.

Fig. 4. Migration of the S antigen in human diploid cells from nucleus to cytoplasm 7 hr after infection.

Fig. 5. After 24 hr most of the S antigen in monkey kidney cells was detected outside the nucleus.

Fig. 6. Also in human diploid cells most of the S antigen was detected outside the nucleus after 24 hr.