ROLE OF ts MUTANTS OF INFLUENZA VIRUS IN THE DEVELOPMENT OF A PERSISTENT INFECTION OF MDCK CELLS

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Temperature-sensitive mutants of various RNA viruses are known to participate in persistent infections of cell cultures (*1*). To our knowledge, selection of ts mutants of influenza virus persisting in cell cultures has not been reported.

In the course of 158 days we studied the persistence of A/Victoria/35/72 (H3N2) influenza virus in cultures of susceptible Madin-Darby canine kidney (MDCK) cells. The persistent infection was induced and the persisting viruses were isolated as described (*2*).

Lowering of incubation temperature of infected cells from 37 to 34 C led to a markedly enhanced virus reproduction. On days 20, 32 and 158 of a persistent infection of cells incubated at 37 °C, no cytopathic effect (CPE) and no haemagglutinin (HA) in the culture fluid was demonstrated; the infectious virus titres on days 20 and 32 were 2 and 1 log EID_{50}/0.2 ml, respectively and on day 158 no infectious virus was detected in either the culture fluid or the cells. A temperature shift from 37 to 34 °C on days 20 and 30 and incubation at 34 °C for 48 hr led to the appearance of a moderate CPE and HA in the culture fluid (titre of 2); the titres of virus in the culture fluid increased to 5.5 and 6.0 log EID_{50}/0.2 ml on days 20 and 32, respectively. On day 158, the temperature shift did not lead to the appearance of a CPE or HA, but infectious virus was detected in the cell homogenate.

In studying the thermosensitivity of reproduction of the persisting viruses isolated on days 9, 14, 18, 33, 43, 52, 62 and 158, we found that all reproduced in chick embryos at 34 °C to titres of 8 log EID_{50}/0.2 ml but at 40 °C only to titres from 1.0—3.5 log EID_{50}/0.2 ml. By contrast, the replication of the original wild type virus remained practically unchanged at 40 °C (titre of 8.5 log EID_{50}/0.2 ml).

Cloning of the persisting viruses by the plaque method in MDCK cells made it possible to study the course of accumulation of ts mutants in the population of persisting viruses. The original A/Victoria/35/72 virus, used for induction of the persistent infection, contained no ts clones. On day 9, the persisting virus population contained 31.5% ts clones, which proportion increased to 66.7% on day 14. On days 62 and 158, the whole population of the persisting virus consisted of ts clones.

Irrespective of the fact that no ts clones were demonstrated in the original virus, persistence of influenza virus in MDCK cells was accompanied by the selection of ts mutants which, at day 62 of the persistent infection, completely suppressed the wild ts^+ clones. The nature of the phenomenon remains obscure. According to Youngner *et al.* (*3*), in persistent infection of L cells with vesicular stomatitis virus, selection of ts mutants is due to their interfering activity in respect to wild type virus.

References

1. Rima, B. K., and Martin, S. Y., *Med. Microbiol. Immunol.* **162**: 89, 1976.
2. Golubev, D. B., and Medvedeva, M. N., *J. Hyg. Epidem.* (*Praha*) **22**: 23, 1978.
3. Youngner, Y. S., Preble, O. T., and Jones, E. V., *J. Virol.* **28**: 6, 1978.