Comparative Production of Paramyxoviruses by Avian Heart Cells

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SUMMARY

Infection of chicken embryonic heart, lung and liver cells with parainfluenza 1 and 3 viruses revealed that the most susceptible (S) cell is the heart cell and the least susceptible (LS) cell is the liver cell. The results of these experiments are similar to observations where the S and LS cell for another paramyxovirus, mumps virus, were also cultivated from the chicken embryonic heart and liver, respectively.

Previous observations in our laboratory have established that chicken embryonic heart cells consistently produce more mumps virus than cells cultivated from other chicken organs (Davis & St Geme, 1969). Mumps virus can also be recovered in highest titre from the intact chicken heart throughout incubation in ovo following inoculation of the embryo at 12 to 16 h of age (St Geme et al. 1973b). The lowest titres of virus are found in the liver and cultivated liver cells produce the least amount of mumps virus. Lung tissue and lung cells occupy an intermediate point in this pattern of mumps virus replication in ovo and in vitro. Thus, the correlation between replication of mumps virus in chicken embryonic organs and differentiated cells cultivated from these tissues is rather precise. We have designated the most susceptible heart cell as the S cell and the least susceptible liver cell as the LS cell.

The biological determinants of virus synthesis by the S and LS cell do not include early events in the interaction between virus particle and cell (Davis, Dufour & St Geme, 1971). The rates of attachment to and penetration of mumps virus into the S and LS cell are similar. Neither S nor LS cells produce detectable interferon following mumps virus infection. Non-interferon regulator proteins may attenuate virus production by LS cells, highlighting the most important difference between the S and LS cell which is more efficient release of virus particles by the heart cell (St Geme et al. 1973a). Biochemical analysis of plasma membranes of the S and LS cell has shown that the lipid composition of heart cell membranes may explain the superior maturation, enveloping and release of mumps virus by S cells, as Klenk & Choppin (1970) have shown to be true for another paramyxovirus, parainfluenza SV5 virus, in the high virus-producing monkey renal cell and the low virus-producing hamster renal cell.

The observation that the lipid composition of plasma membranes may determine the susceptibility of vertebrate cells to two paramyxoviruses prompted our evaluation of the replication of parainfluenza 1 and 3 viruses in chicken embryonic cells. If, indeed, membrane lipid structure is the common denominator of cellular susceptibility to paramyxoviruses, one might predict that the S and LS cell for these two paramyxoviruses is also the heart and liver cell, respectively.

Heart, lung and liver were removed from 12-day-old chicken embryos, minced, rinsed free of blood, and trypsinized with several changes of 0.1 to 0.2 % trypsin solution for 45 min. After low-speed sedimentation, cells were resuspended in growth medium consisting of Eagle's basal medium using Hanks' balanced salt solution and supplemented with 10 % foetal bovine serum and 100 units penicillin and 100 µg streptomycin per ml. Singly dispersed cells were diluted so that 10⁶ cells per ml could be added to glass culture tubes, yielding nearly confluent monolayers of approx. 3 x 10⁶ cells per tube after one day, when they were
infected with parainfluenza virus. Organotypic reaggregation of freshly cultivated embryonic cells imparted to the heart, lung and liver cultures the histological appearance of the corresponding intact organs. Evidence of continued cellular differentiation for the duration of the experiments was provided by rhythmic contraction of heart cells and the presence of glycogen granules in liver cells.

Parainfluenza 1 virus (HVJ-MN), Sendai, was obtained from Dr Hideo Fukumi, Tokyo, as the 8th passage in HeLa cells. The 12th and 13th passage in HeLa cells, made in our laboratory, served as the stock virus for these experiments. Parainfluenza 3 virus (C243), from the American Type Culture Collection, was propagated in HeLa cells for the first time in our laboratory and the 5th and 6th passages in HeLa cells were used in these experiments. Both viruses produced clearly discerned haemadsorption plaques in HeLa cell monolayers under liquid medium, using 0.1% guinea pig erythrocytes and incubation at 4 °C for 4 h. The optimal time for quantitation of the number of parainfluenza 1 virus plaques was 72 h after inoculation of monolayers, and 48 h for parainfluenza 3 virus.

Cells were inoculated with 1 to 10 p.f.u./cell and liquid medium was harvested at the time of infection and at daily intervals thereafter. Figures 1 and 2 depict the results of representative experiments. Heart cells produced substantially more parainfluenza 1 and 3 virus than liver cells. Averaging many experiments and intervals of sampling, approx. fivefold more parainfluenza 1 virus was produced by heart cells. The differential capability of heart and liver cells to replicate paramyxoviruses was more striking in experiments with parainfluenza 3 virus, as heart cells produced greater than 50-fold more virus than liver cells.

Although for purposes of visual simplicity and because of inconsistent experimental data the pattern of virus replication is not illustrated in the figures, lung cells produced as much and on some occasions more parainfluenza 1 virus than heart cells. In contrast, lung cells produced an intermediate amount of parainfluenza 3 virus in some experiments and in others produced no more virus than liver cells. As mentioned above, lung cells are also intermediate producers of mumps virus and in the exceptional experiment synthesized almost as much virus as heart cells.

Paramyxovirus infection of cells cultivated from chicken embryonic organs indicates that the heart cell is consistently the S cell for mumps virus, parainfluenza 1 virus, and parainfluenza 3 virus, and in equally consistent contrast the LS cell for these three viruses is the liver cell. The composition of genome and virus proteins of these three paramyxoviruses differs (Brostrom, Bruening & Bankowski, 1971; East & Kingsbury, 1971; Mountcastle, Compans & Choppin, 1971). Our experiments with mumps virus suggest that capsid glycopeptides which permit attachment and perhaps penetration do not determine differential susceptibility of chicken cells (Davis et al., 1971). If the structural proteins of these three antigenically interrelated viruses are similar, and these structural proteins exercise a crucial role in the release of virus particles, one might predict that in any particular virus-host cell system the S cell would be the same for all viruses in the same taxonomic group or subgroup. However, this hypothesis fails to explain the presence of the consistent LS cell. The pattern of paramyxovirus replication in chicken embryonic heart, lung and liver cells supports the contention that the host cell determines virus tropism, that the crucial phase of interaction is enveloping and release of virus particles, and in some obscure fashion the higher molar ratio of cholesterol to phospholipid and the higher phosphatidylethanolamine and lower phosphatidylcholine content in the plasma membrane permit cells from one organ to complete virus replication more efficiently than cells from other tissues (Klenk & Choppin, 1970; St Geme et al. 1973a).
The flaw in these studies is the inability of parainfluenza 3 virus to multiply in the tissue of chicken embryos inoculated with virus during the first 24 h of incubation \textit{in ovo}. Parainfluenza 1 virus does multiply in embryonic tissues. However, the inoculation of minimal concentrations of virus killed embryos within one week, precluding the careful quantitation of virus in individual organs. Thus, the S and LS chicken embryonic organ for parainfluenza 1 virus is unknown, and for the C243 strain of parainfluenza 3 virus does not exist. In contrast, the correlation between the replication of mumps virus \textit{in ovo} and \textit{in vitro} is very good.
Short communications

Mumps virus replicates in heart tissue and heart cells to high titre, whereas the replication of virus in liver tissue and liver cells is low (Davis & St Geme, 1969; St Geme et al. 1973b).

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Department of Pediatrics
Harbor General Hospital
UCLA School of Medicine
1000 W. Carson Street
Torrance, California 90509 U.S.A.

J. W. ST GEME JUN.
CATHERINE W. C. DAVIS
H. L. MARTIN

REFERENCES


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