Protein Synthesis in Vero Cells Abortively Infected with Influenza B Virus

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SUMMARY

Growth of influenza B virus was found to be greatly restricted in Vero cells compared with that in MDCK cells. The analysis of protein synthesis in infected cells showed that the synthesis of M protein is selectively inhibited in abortive infection. Cell fractionation experiments demonstrated that the viral glycoproteins, HA and NA, migrate from rough membranes via smooth membranes to plasma membranes in abortive as well as in productive cells. These results suggest that intracellular migration of influenza B virus glycoproteins occurs independently of the synthesis of M protein although M protein synthesis appears to be required for the formation of virus particles.

It is well known that, depending on the host cell and the virus strain used, replication of influenza virus may be abortive with respect to the formation of virus particles. Although a considerable amount is known about abortive infection by influenza A virus (Lerner & Hodge, 1969; Sugiura, 1972; Bosch et al., 1978; Valcavi et al., 1978; Caligiuri & Holmes, 1979), a biochemical analysis of abortive infection by influenza B virus has not yet been reported. In the present study we have analysed the abortive infection of Vero cells by influenza B virus as compared to productive infection of MDCK cells.

The Lee/40 strain of influenza B virus was grown in the allantoic cavity of 10-day-old embryonated hen’s eggs at 35 °C for 48 h and the allantoic harvest was used for infection of cells. The MDCK line of canine kidney cells and the Vero line of African green monkey kidney cells were grown in Eagle’s minimal essential medium supplemented with 10% bovine serum. When the growth of influenza B virus was compared in MDCK and Vero cells under single cycle conditions, MDCK cells were found to be highly productive in the formation of virus particles, i.e., the haemagglutination titre of media rose as high as 1024 HAU/ml after incubation at 37 °C for 24 h. On the other hand, no haemagglutination activity was detectable in the media of Vero cells even after a prolonged incubation up to 48 h post-infection.

To investigate the synthesis of individual virus-specific proteins in abortive and productive cells, cells were infected with influenza B virus at a multiplicity of 20 to 50 EID<sub>50</sub>/cell and then labelled at 37 °C for 30 min with [35S]methionine (5 μCi/ml) at 7 h after infection. The polypeptides synthesized in the cells were analysed by SDS–polyacrylamide gel electrophoresis according to the method of Laemmli (1970), using 13% gels (acrylamide:bis-acrylamide at 218.5:1) containing 4 M-urea. The protein pattern of infected MDCK cells shown in Fig. 1(a) clearly demonstrates the synthesis of HA, NA, NP, NS<sub>1</sub> and M in these cells. Although only one P protein can be seen in this gel pattern, three P proteins were resolved in some other experiments (see Fig. 2). In addition to these proteins, a protein designated NP<sub>e</sub> which migrates slightly faster than NP was resolved. This protein appeared to be a cleavage or degradation product of NP since when the homogenates of infected cells were incubated at 37 °C, NP was rapidly converted to this protein and this conversion was completely inhibited by addition of protease inhibitors (data not shown). The gel pattern of infected Vero cells (Fig. 1a) shows that while most virus-specific polypeptides are synthesized...
in these cells in proportions similar to MDCK cells, the synthesis of M protein was not detectable in Vero cells. To rule out the possibility that the background, due to incorporation of label into host cellular proteins, may have obscured the band corresponding to M protein, the immunoprecipitates obtained after treatment of infected cells with anti-rabbit serum against egg-grown virions were analysed by gel electrophoresis as described by Saleh et al. (1979). The results shown in Fig. 1 (b) indicate that a trace amount of M protein was synthesized in Vero cells, but the amount was much less than in MDCK cells.

Previous cell fractionation studies with influenza A virus have revealed that viral glycoproteins are synthesized in association with rough membranes and migrate through smooth membranes to plasma membranes (Compans, 1973; Hay, 1974; Klenk et al., 1974; Meier-Ewert & Compans, 1974). In addition, Lohmeyer et al. (1979) have recently demonstrated that intracellular migration of influenza A viral glycoproteins occurs independently of M protein synthesis. To determine if this is also the case with influenza B virus, the distribution of virus proteins between subcellular fractions was compared between MDCK and Vero cells. Fig. 2 (a) shows the protein patterns of smooth and rough membranes isolated from infected cells. The distribution of HA glycoproteins into smooth and rough membrane fractions was similar in Vero and MDCK cells, which suggests that the migration of HA from rough to smooth membranes normally proceeds in Vero cells. Fig. 2 (b) shows the polypeptide profiles of plasma membranes isolated from infected cells. Plasma membranes of Vero cells as well as of MDCK cells contained glycoproteins, HA and NA as major components although the cleavage products of HA polypeptides, HA$_1$ and HA$_2$, were found
Fig. 2. Association of virus proteins with intracellular membranes and plasma membranes of MDCK and Vero cells. Cells were labelled at 37 °C with [35S]methionine for 2 h at 7 h post-infection. (a) Smooth and rough membranes were isolated as described by Caliguiri & Tamm (1970). Lane 1, Smooth membranes of MDCK cells; lane 2, rough membranes of MDCK cells; lane 3, smooth membranes of Vero cells; lane 4, rough membranes of Vero cells. (b) Plasma membranes were isolated according to the procedures described previously (Nakamura & Compans, 1977). Lane 1, MDCK cells; lane 2, Vero cells.

in plasma membranes of MDCK cells but not in those of Vero cells, suggesting that incorporation of virus glycoproteins into plasma membranes is not affected by the amount of M protein synthesized in cells. Thus, it is concluded that intracellular migration of influenza B virus glycoproteins does not depend on the synthesis of M protein, as has been observed with influenza A virus (Lohmeyer et al., 1979).

The data presented here showed that the most striking difference between permissive MDCK and non-permissive Vero cells infected with influenza B virus was the marked reduction in the synthesis of M protein in the latter cells. Similar observations have been made in studies on abortive infection of influenza A viruses (Bosch et al., 1978; Valcavi et al., 1978; Lohmeyer et al., 1979). However, we are not certain if the defective M protein synthesis is the only cause for abortive infection in Vero cells. Caliguiri & Holmes (1979) have demonstrated that the restriction of influenza A virus replication in HeLa cells is not due to a specific defect in the synthesis of virus proteins but due to a host cell-dependent defect in the final stage of bud formation at plasma membranes. We cannot rule out the possibility that this mechanism is also involved in abortive infection in our system. Several previous studies have shown that a lack of virus assembly in abortive infection of influenza A virus is the result of a block in the transport of NP out of the nucleus (Kelly & Dimmock, 1974). It is, however, unlikely that this mechanism acts in abortive infection in the system analysed here. There was no significant difference in the amounts of NP in cytoplasmic fractions of MDCK and Vero cells (see Fig. 2a).
The reason for the defect in M protein synthesis in Vero cells is still not understood. Bosch et al. (1978) have presented evidence that the reduction in M protein synthesis in abortive cells results from reduced production of its mRNA. However, it is also possible that the block in M protein synthesis may be a result of some other defect(s) during the early events in the replication cycle since the synthesis of M protein was found to be a late event in replication of influenza B virus (data not shown), as has been established with influenza A virus (Hay, 1974; Meier-Ewert & Compans, 1974). In considering the mechanism of inhibition, it is to be noted that the defect in M protein synthesis was also observed in Vero cells infected with a recently isolated influenza B strain, B/Yamagata/26/77, while normal amounts of M protein were detected in cells infected with influenza A/WSN/33 or A/FM/1/47 strain (data not shown), suggesting that the reduced synthesis of M protein in Vero cells may be specific for influenza B virus.

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REFERENCES


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