An Interference Phenomenon Associated with a Measles Virus SSPE Isolate (Halle)

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SUMMARY

A measles virus (Halle SSPE isolate) induced ring plaque phenomenon (concentric rings of living and dead cells) has been shown to be associated with the temperature-dependent production of interfering particles. The interfering particles have been purified by potassium tartrate linear gradient centrifugation and have buoyant densities of 1.15 g/ml (M particle) and 1.06 g/ml (L particle) respectively. Both interfering particle populations have been shown to decrease the yield of wild-type measles virus from infected cells by 50% when co-infection experiments were performed. Neither the M or L particle population interfered with the growth of VSV in the same host-cell system.

Measles virus (MV), a member of the Paramyxoviridae family (genus Morbillivirus) is currently undergoing extensive investigation, particularly with regard to its possible involvement in the aetiology of certain neurological diseases and/or disorders (ter Meulen & Hall, 1978). Several measles-like viruses (strains?) have been isolated by laboratory co-cultivation techniques, from cases of subacute sclerosing panencephalitis (SSPE) (Horta-Barbosa et al., 1969; Payne et al., 1969).

During an investigation of the growth properties of an MV SSPE isolate, designated Halle (MVH), a temperature-dependent plaquing phenomenon (termed ring plaques) was observed. A study of the ring plaque growth phenomenon led to the isolation of MV interfering particles. This communication describes and discusses the possible implications of the phenomenon observed.

The MVH isolate (obtained from Dr T. F. Wild, INSERM, Lyon, France) was twice plaque-purified in cultures of Vero cells (ATCC CCL81; Flow Laboratories). As many laboratories have noted that certain strains of MV grow to higher titres at 32 °C than at 37 °C (T. F. Wild, personal communication; A. Salmi, personal communication; this study), the MVH isolate was grown at 32 °C, 37 °C and 39 °C and examined at regular intervals for plaque production. At 5 days post-infection, the virus grown at 37 °C showed plaques with clear centres surrounded by a ring of cells (Fig. 1). As incubation progressed (up to 20 days post-infection) more rings appeared as the plaques enlarged until a concentric ring pattern appeared (ring plaques). Vital dye (neutral red) staining indicated that the ring plaques consisted of alternating, concentric rings of living and dead cells. Plaques observed at 32 °C and 39 °C were distinct from those seen at 37 °C (Fig. 1) and at no time were the characteristic ring plaques seen at 32 °C or 39 °C. Use of alternate host-cell systems for plaque titration of the MVH isolate (BSC-1, ATCC CCL26; HEp-2, ATCC CCL23; KB, ATCC CCL17) gave similar results, although the ring plaques produced were considerably smaller in size (not shown). Such results would indicate that the phenomenon is not host-cell dependent. The larger plaque size observed in the Vero host-cell system can be related to the greater efficiency of MV replication in that particular cell system. Plaque titration of the MV LEC SSPE isolate and MV Edmonston strain (obtained from Dr E. Norrby, Karolinska Institute, Stockholm, Sweden), under the same conditions as that used for the production of the MVH ring plaques, did not produce the same phenomenon.
Fig. 1. Appearance of plaques produced by measles virus (Halle SSPE isolate) on Vero cell monolayers. (a) Incubation at 37 °C for 12 days; (b) incubation at 32 °C for 18 days; (c) incubation at 39 °C for 7 days.

In order to further examine the MVH isolate associated with ring plaque production, large amounts of MVH virus were prepared by infecting Vero cell monolayers at 37 °C in roller bottles at a multiplicity of 0.05 p.f.u./cell. At 72 h post-infection, when 90% c.p.e. was observed, the infected cells were frozen by placing the roller bottles at −35 °C. Virions were purified using a modification of the technique of Hall & Martin (1973, 1974). The crude virus suspension obtained was layered on to a 15 to 50% linear gradient of potassium tartrate in 10 mM-tris–HCl, containing 1 mM-EDTA at pH 7.4, and centrifuged at 155000 g for 16 h in a Beckman SW40 Ti rotor. At the completion of the centrifugation period, 15-drop fractions were collected from each tube and tested for haemagglutinating activity (a standard microtitre system using 0.5% final concentration Cercopithecus aethiops erythrocytes and 37 °C incubation was used) and infectivity.

Following centrifugation of the MVH preparations in potassium tartrate gradients, three distinct particle populations were observed at buoyant densities (g/ml) of 1.225, 1.15 and 1.06. The three particle populations were designated as H (heavy, complete), M (middle, incomplete) and L (light, incomplete) respectively. Haemagglutinating and infectivity assays revealed that all such activity was associated only with the H particle population. Maximum haemagglutinating titres (16- to 32-fold higher) were obtained when assays were carried out in the presence of 800 mM-ammonium sulphate (salt-dependent haemagglutinin; Schleuderberg & Nakamura, 1967).

The interfering property of the M and L particles with the growth of the H particle, MVH and other MV strains and isolates (Edmonston and LEC) was determined by adding aliquots of the gradient-purified particles to susceptible cell monolayers with, or before, the addition of a standard inoculum of test virus. The infected cells were incubated for 12 days at 37 °C. Interference was judged to have occurred if a 50% or greater reduction in titre of the test
Table 1. **Interference with growth of measles virus (Halle isolate)**\* by \(M\) and \(L\) particles purified by potassium tartrate linear gradient centrifugation

<table>
<thead>
<tr>
<th>Virus</th>
<th>Plaques/plate†</th>
<th>Interference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVH only</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>VSV only‡</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>(M) particle only§</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>+ MVH, 0 h p.i.</td>
<td>44</td>
<td>0</td>
</tr>
<tr>
<td>+ MVH, 6 h p.i.</td>
<td>20</td>
<td>55</td>
</tr>
<tr>
<td>+ MVH, 16 h p.i.</td>
<td>21</td>
<td>52</td>
</tr>
<tr>
<td>+ VSV, 6 h p.i.</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>(L) particle only§</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>+ MVH, 0 h p.i.</td>
<td>44</td>
<td>0</td>
</tr>
<tr>
<td>+ MVH, 6 h p.i.</td>
<td>22</td>
<td>50</td>
</tr>
<tr>
<td>+ MVH, 16 h p.i.</td>
<td>21</td>
<td>52</td>
</tr>
<tr>
<td>+ VSV, 6 h p.i.</td>
<td>40</td>
<td>0</td>
</tr>
</tbody>
</table>

\* The Edmonston and LEC strains of MV were also used in interference assays with results obtained being similar to that with MVH.

† Inoculum diluted to contain a countable number of plaques/plate.

‡ VSV was included in the interference assays to determine if the interference observed was a non-specific phenomenon.

§ 0.1 ml of gradient-prepared material was added to each plate.

‖ p.i., Post-infection.

virus was observed. Table 1 gives the results obtained. Both the \(M\) and \(L\) particles interfered with the normal production of the MVH, Edmonston and LEC virus plaques. Non-specific inhibition of virus production did not appear to be involved in the phenomenon, as a VSV preparation assayed under the same conditions produced ‘normal’ amounts of infectious virus. The induction of the interference phenomenon was observed to have occurred within 6 h of the addition of either the \(M\) or \(L\) particles to cell cultures (at which time an aliquot of test virus was added). As a detailed biochemical examination of the interference phenomenon has not been done, no definitive comment can be made as to the mechanism involved.

It is well known that undiluted passage of certain viruses results in the cyclic increase and decrease of infectious (complete) virus and non-infectious (incomplete) interfering particles (Huang, 1973). The ring plaque phenomenon described in this study is a graphic illustration of the cyclic phenomenon. The clear, non-stained areas of the ring plaque represent the cycle of maximum complete infectious particle production, while stained, living cell areas represent that portion of the cycle where infectivity is low and (defective-) interfering particle production is high. The occurrence of ring plaques at 37 °C would indicate that low virus titres observed in this and other laboratories may be the result of the presence of large numbers of interfering particles.

That MV interfering particles do occur is shown by the appearance of the two light buoyant density \(M\) and \(L\) particle populations in potassium tartrate gradients. The possibility does exist, however, that the \(M\) and \(L\) particles observed do not consist of intact virus-like particles, but instead consist of subviral components or aggregates. This possibility cannot be resolved by electron microscopy, as the conditions of negative-contrast staining could disrupt fragile defective particles which would then resemble membrane fragments. No explanation is yet available to explain the light buoyant density of the \(L\) particle. Hall & Martin (1974) have previously described the appearance of defective-interfering particles of MV. Whether or not the \(M\) and \(L\) particles described in this study are, in fact, true defective-interfering particles will not be known until more information is obtained concerning the nature of the associated RNA species (Huang, 1973).

The appearance of ring plaques has been previously described in association with the production of infectious pancreatic necrosis virus plaques (IPNV) (Macdonald, 1978) and
lymphocytic choriomeningitis virus (LCMV) (Pfau et al., 1973). In the case of IPNV, the ring plaques consisted of a single diffuse area surrounding a central plaque and were shown to be associated with the presence of IPNV defective-interfering particles (not isolated). LCMV-produced ring plaques were shown to be related to the pCO₂ of the growth atmosphere, with a possible relationship of the pCO₂ and interfering particle production having been postulated by the authors (Pfau et al., 1973).

Further experiments are now under way to determine the mechanism whereby the temperature-dependent production of the M and L particles may be involved in the interference phenomenon observed, and in in vivo models of persistent MV infections.

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REFERENCES


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