Phenotypic Mixing between Murine Oncoviruses and Murine Cytomegalovirus

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SUMMARY

In vitro interactions between murine cytomegalovirus (MCMV) and murine leukaemia viruses (MuLV), two groups of enveloped viruses capable of causing persistent or latent infections in vivo, were examined for evidence of phenotypic mixing. The growth of MCMV in murine cells productively infected with ecotropic MuLV was shown to result regularly in the production of phenotypically mixed particles having the envelope antigens of MuLV and the genome of MCMV [MCMV(MuLV) pseudotypes]. The identity of such pseudotype particles was confirmed by the use of specific anti-MuLV serum and by the demonstration of restriction due to viral interference of penetration of these particles on MuLV-infected murine cells. This restriction was independent of N- or B-tropism. The production of reverse pseudotypes could not be examined because of the lytic effects of MCMV on the requisite assay cells.

Phenotypic mixing of virus glycoproteins between both related and unrelated enveloped viruses is a well-recognized in vitro phenomenon that may have significance in vivo, particularly in interactions between different endogenous viruses or between an endogenous virus and an exogenous one introduced by means of an acute infection (Hanafusa et al. 1964; Huebner et al. 1966; Vogt, 1967; Fischinger & O'Connor, 1969; Huang et al. 1974; Gabelman et al. 1975; Zavada, 1976; Besmer & Baltimore, 1977; Ishimoto et al. 1977; Levy, 1977a, b; Schnitzer et al. 1977; Weiss & Wong, 1977; Schnitzer, 1979). Murine cytomegalovirus (MCMV), a member of the herpesvirus group, and murine oncoviruses are both known to be capable of infecting mice and producing persistent or latent infections, and both groups of viruses have been implicated, directly or indirectly, in the production of tumours or the transformation of cells (Gross, 1970; Weller, 1971; Henson et al. 1972; Gardner et al. 1974; Ablashi et al. 1976; Michelson-Fisk, 1977). It therefore was of interest to examine possible in vitro interactions between these two viral groups to look for evidence of phenotypic mixing, since pseudotype formation could alter the spread of these viruses and be of importance in their pathogenesis.

Mouse cells, both uninfected (NIH/3T3, Balb/3T3) and productively infected with N (AKR virus Gross passage A)- or B (WN 18023)-tropic murine leukaemia virus (MuLV), respectively (NIH-MuLV, Balb-MuLV; kindly supplied by N. Teich, ICRF, London), were used in this study. Infection of these cells with MCMV, Smith strain (kindly supplied by C. A. Mims, Guy's Hospital, London) was carried out at a multiplicity of 1 to 2 p.f.u./cell. The virus was allowed to adsorb for 1 h at 37 °C and the cells were then washed twice with phosphate-buffered saline (PBS) and fed with Dulbecco's modified essential medium (DMEM) supplemented with 10% foetal calf serum. After 72 h incubation at 37 °C, at which time the infected cells showed a 3 to 4+ cytopathic effect, the supernatant was removed and clarified by centrifugation at 2000 rev/min for 10 min in a bench-top MSE
Fig. 1. Production of MuLV pseudotypes of MCMV and their host range for penetration. MCMV harvests from the indicated MuLV-infected and uninfected murine cells were titrated on the indicator cells shown both prior to (open bars) and after (stippled bars beneath arrows) treatment with anti-MCMV serum. Non-neutralizable MCMV infectivity (stippled bars) represents MCMV-(MuLV) pseudotype titre. NT, Not tested.

centrifuge. Samples were taken for neutralization or to be titrated directly. The inoculum of 0.2 ml per 30 mm dish was allowed to adsorb for 60 min at room temperature, the cells were then washed twice with PBS, overlaid with 1.5% agar medium and incubated at 37 °C for 4 days before being fixed in formalin and stained with crystal violet. Neutralization was performed by mixing samples of virus with heat-inactivated immune serum having activity against MuLV or MCMV. The latter serum was prepared by injecting rabbits intramuscularly and intravenously once a week for 10 weeks with culture medium from MCMV-infected rabbit kidney cells and the rabbits were then bled 4 weeks after the last injection. The anti-MuLV serum was a gift from N. Teich. Titrations of both non-neutralized and neutralized
Table 1. Effect of different antisera on the infectivity of MCMV

<table>
<thead>
<tr>
<th>Cell used for growth</th>
<th>No antiserum</th>
<th>Anti-MCMV</th>
<th>Anti-MCMV*, anti-MuLV</th>
<th>Anti-MuLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balb/3T3</td>
<td>5.3</td>
<td>&lt; 1.0</td>
<td>NT†</td>
<td>NT</td>
</tr>
<tr>
<td>Balb-MuLV</td>
<td>5.3</td>
<td>&lt; 1.9</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>NIH/3T3</td>
<td>5.8</td>
<td>&lt; 1.0</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>NIH-MuLV</td>
<td>5.1</td>
<td>&lt; 1.0</td>
<td>5.1</td>
<td></td>
</tr>
</tbody>
</table>

* Log_{10} p.f.u./ml on 1° Balb cells.
† Not tested.

virus were performed in parallel on all indicator cells employed [primary (1°) Balb mouse embryo cells, Balb/3T3, Balb-MuLV, NIH/3T3, NIH-MuLV].

The results of these studies are shown in Fig. 1. MCMV infection of mouse cells not known to be producing ecotropic MuLV (Balb/3T3, NIH/3T3) resulted in the production of 10^5 to 10^6 p.f.u./ml of MCMV, the titre being largely independent of the cell type used for the plaque assay. Treatment of such progeny virus with antiserum to MCMV at a final dilution of 1:15 for 1 h at room temperature completely neutralized all infectivity. MCMV infection of the Balb/3T3 cells producing B-MuLV (Balb-MuLV), resulted in the production of similar levels of infectious MCMV. Treatment of this progeny virus with the same concentration of antiserum (1:15 dilution), known to be adequate for complete neutralization of MCMV harvests from murine cells not producing MuLV, resulted in the demonstration of residual infectivity when such treated harvests were assayed on uninfected 1° Balb, Balb/3T3 or NIH/3T3 cells. Addition of further anti-MCMV serum or use of higher antiserum concentrations for neutralization did not alter these results (data not shown). Comparable data could be demonstrated after MCMV infection of uninfected NIH/3T3 and MuLV-infected NIH/3T3 cells respectively. The reason for the diminished growth of MCMV on the MuLV-infected NIH cells, as well as the higher proportion of residual infectivity (pseudotype fraction > 1%) is not known but may be related to the fact that the infected NIH cells were producing a higher level of oncovirus (measured by reverse transcriptase activity) when compared to the Balb-MuLV cultures.

These results demonstrated that one consequence of the growth of MCMV in MuLV-infected murine cells is the production of a proportion of infectious progeny virions having an MCMV genome but not capable of neutralization by antiserum to MCMV. Since virus infectivity is a consequence of interactions between virus glycoproteins and specific cell surface receptors (Weiss, 1976), this suggested that the residual MCMV infectivity could be a consequence of phenotypic mixing between MCMV and MuLV with the production of pseudotype particles having the genome of MCMV but the envelope antigens of MuLV. To confirm this hypothesis, two types of experiment were performed. Progeny virus from MuLV-producing cells infected with MCMV was treated in parallel with antiserum to MCMV alone (final dilutions 1:15) and with a mixture of antisera to both MCMV (final dilution 1:15) and MuLV. While treatment of this presumably mixed virus population with the antiserum to MCMV alone always resulted in the demonstration of residual MCMV infectivity, addition of the anti-MuLV serum regularly eliminated all remaining p.f.u. (Table 1). Treatment of these virus harvests with anti-MuLV serum alone, even when the MCMV titre was reduced to 10^5 MCMV p.f.u./ml, failed to demonstrate any neutralization of MCMV infectivity, demonstrating that the loss of infectivity was not due to a non-specific effect of the anti-MuLV serum on the remaining fraction of infectious particles.

The presence of pseudotype particles bearing MuLV glycoproteins in these harvests was
further confirmed by the demonstration of specific viral interference. From previous studies utilizing pseudotype particles (Zavada, 1972; Krontiris et al. 1973; Besmer & Baltimore, 1977), it has been demonstrated that murine cells producing ecotropic MuLV are resistant to infection by exogenous ecotropic MuLV due to a block at the level of virus penetration. This block is specific for ecotropic MuLV and independent of N- or B-tropism (Sarma et al. 1967; T. J. Schnitzer, unpublished observations). Similar interference was demonstrated in these studies (Fig. 1). Treatment of progeny virus from MCMV-infected, MuLV-producing murine cells with anti-MCMV serum regularly resulted in the demonstration of residual MCMV infectivity only when neutralized harvests were assayed on uninfected murine cells. When the same indicator cells were pre-infected with N- or B-tropic MuLV, no residual MCMV infectivity could be demonstrated despite the fact that MCMV itself produced plaques with equal efficiency in MuLV-infected and uninfected murine cells.

Pseudotype particles possessing the genome of MCMV and the surface glycoproteins of MuLV are, therefore, a direct consequence of the infection of murine cells producing MuLV. Whether actual MuLV production is required, or if simple expression of MuLV glycoprotein on the cell surface is sufficient for pseudotype production, was not investigated but the latter is suggested by studies with VSV and oncoviruses in other systems (Love & Weiss, 1974; Weiss et al. 1975). Similarly, the production of 'reverse' pseudotype particles having the genome of MuLV and the surface glycoprotein of MCMV was not examined due to the fact that the non-neutralized pure MCMV produced was lytic for the cells used to assay the MuLV genome.

The demonstration in these studies of in vitro phenotypic mixing between murine oncoviruses and another naturally-occurring latent infectious agent of mouse, MCMV, suggests that in vivo interactions between these two agents is possible and may be of pathogenic importance. The ability of MCMV to acquire MuLV glycoprotein could permit introduction of this genome into cells otherwise resistant to MCMV infection. The presence of this genetic information may then alter cellular functions in a manner sufficient for the expression of pre-existing malignant potential. Alternatively, the possibility of the production of pseudotypes having an MuLV genome and MCMV coat from cells doubly infected by these viruses could result in infection and subsequent transformation of cells normally resistant to MuLV owing to the extremely broad adsorption and penetration capacity of MCMV (vide supra; Kim & Carp, 1971; Gonczol et al. 1978). Further studies of these agents, both in vitro and in vivo, would therefore be of importance to better define such viral interactions and to examine their significance.

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REFERENCES


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