Studies on Intracellular and Membrane Antigens
Induced by Marek’s Disease Virus

(Accepted 21 June 1973)

SUMMARY

Prolonged passage of Marek’s disease virus in cell culture resulted in attenuation of the virus and inability of the virus to produce the membrane antigen. However, the apathogenic herpes virus of turkeys produced a serologically similar membrane antigen. Cell culture passage did not cause any change in production of intracellular antigens.

Marek’s disease (MD) is a lymphoproliferative disease of chickens and is caused by a herpes virus (Churchill & Biggs, 1967; Nazerian et al. 1968). Intracellular antigens are detected in cell cultures infected with MD virus (MDV) by the immunofluorescence (IF) technique (Purchase, 1969). Combined IF and electron microscopic examination of infected cells demonstrated that these antigens are virus-related and are only found in cells producing the virus (Nazerian & Purchase, 1970). Intracellular IF antigens were also detected in epithelial cells of the feather follicles and in certain other tissues of infected chickens (Calnek & Hitchner, 1969; Purchase, 1970). A new surface antigen detected (Chen & Purchase, 1970; Ahmed & Schidlovsky, 1972) on the plasma membrane of MDV-infected chick kidney cultures (CKC) was shown to be specific to the virus infection, but not necessarily different from the intracellular antigens.

Data in this report show that MDV apparently loses the capacity to induce membrane antigen in CKC as it is serially passaged in cell culture and becomes attenuated. The possible significance of this finding is discussed.

The JM and GA strains of MDV were used in this study. These viruses were originally propagated in duck embryo fibroblast (DEF) cultures and were further passaged in either DEF or chicken embryo fibroblast cultures. The pathogenicity of these viruses was assayed by inoculation of 1-day-old chicks of line 15 × 7 maintained in this laboratory and was based on the development of visceral tumours or neural lesions typical of MD. The FC-126 isolate of the herpes virus of turkeys (HVT) (Witter et al. 1970), an orphan virus, antigenically related to MDV, was also used. Antisera were prepared in 15 × 7 chickens. Sera were collected from chickens surviving the disease caused by inoculation of either pathogenic GA strain or pathogenic JM strain of MDV. Serum from a rabbit hyperimmunized against partially purified MDV (kindly provided by J. H. Chen) was also used. The indirect IF technique was applied for detection of intracellular and membrane antigens of different strains of MDV and the HVT. Coverslip cultures of CKC were prepared as described by Purchase (1969) and were infected with sufficient doses of virus to induce well separated, distinguishable MDV or HVT microplaques. For detection of intracellular antigens, coverslip cultures were fixed for 30 s in acetone at 4 °C. They were rapidly dried under forced air and washed in phosphate buffered saline (PBS), pH 7.4, to remove traces of acetone. For detection of the membrane antigen, unfixed viable infected cells were used. Both fixed and unfixed cultures were incubated in 1:20 dilution of serum for 30 min at room temperature,
**Table 1. Induction of intracellular, membrane, and the soluble ‘A’ antigens by pathogenic and attenuated strains of Marek’s disease virus (MDV) and the apathogenic herpes virus of turkeys (HVT) in chick kidney cell cultures (CKC)**

1:20 dilution of chicken and rabbit anti-MDV sera were used.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Strain (passage)</th>
<th>Pathogenicity</th>
<th>‘A’ antigen</th>
<th>Membrane antigen</th>
<th>Intracellular antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDV</td>
<td>JM (4)</td>
<td>+</td>
<td>NT*</td>
<td>2+</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>JM (21)</td>
<td>+</td>
<td>+†</td>
<td>2+</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>JM (61)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>JM (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>JM (150)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>GA (17)</td>
<td>+</td>
<td>+</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>GA (168)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4+</td>
</tr>
<tr>
<td>HVT</td>
<td>FC-126</td>
<td>-</td>
<td>+</td>
<td>1+</td>
<td>2+</td>
</tr>
</tbody>
</table>

* Not tested.
† Data on the ‘A’ antigen were kindly provided by Dr W. Okazaki.

then washed in PBS and depending on the type of serum used, they were then incubated in 1:20 dilution of fluorescin conjugated anti-chicken horse γ-globulin or fluorescin conjugated anti-rabbit goat γ-globulin (Roboz Surgical Instrument Co., Inc., Washington, D.C.) for 15 min. Coverslip preparations were then washed in PBS and were mounted in Elvanol (Rodriguez & Deinhardt, 1960) on glass slides. They were then examined with Leitz dark field fluorescence microscope.

Initial studies indicated that the cell culture passaged (over 150 passages) JM strain of MDV failed to produce the membrane antigen in CKC. This virus was previously shown to have lost its pathogenicity for chicken (Nazerian, 1970). In order to substantiate these results, further experiments were done using different strains of MDV at different culture passage level and different pathogenicities and the apathogenic HVT. Results are presented in Table 1. Membrane antigen was present on cells infected with all pathogenic strains of MDV and was also present on cells infected with the apathogenic HVT. On the other hand, this antigen was not detected on cells infected with attenuated viruses, this was due to either the absence of this antigen or its presence in small undetectable quantities. However, cells infected with either pathogenic or attenuated viruses produced intracellular antigens. These results were identical whether anti-MDV (GA strain) chicken serum, anti-MDV (JM strain) chicken serum or anti-MDV (GA strain) rabbit serum were used. Thus, all three types of sera contained antibodies to both intracellular and membrane antigens. One chicken serum raised against a pathogenic isolate of MDV (kindly provided by H. G. Purchase) was found to be negative for the membrane antigen. Using the GA strain infected cells as the antigen, the titre of antibodies to intracellular antigens in the homologous chicken serum was 32-fold greater than that for the membrane antigen (titres of 1:1280 and 1:40, respectively).

The absence of membrane antigen in the presence of intracellular antigens in cells infected with attenuated strains of MDV and the difference in antibody titres to these antigens indicate that they are probably two different antigens. The membrane antigen may be related to the ‘A’ antigen (Churchill, Chubb & Baxendale, 1969) detected in immunoprecipitin test, since both are lost as the virus is serially passaged in cell culture and becomes attenuated. Thus, strains of MDV positive for the membrane antigen are positive for the ‘A’ antigen and conversely, those negative for the membrane antigen are also negative for the soluble ‘A’ antigen (Table 1). However, induction of the membrane antigen and the soluble ‘A’ antigen
cannot be related to the pathogenicity of MDV since HVT is positive for both antigens while it is completely apathogenic.

**REFERENCES**


*(Received 23 May 1973)*