Murine Cytomegalovirus: Reactivation in Pregnancy

By J. J. Gould and C. A. Mims
Department of Microbiology, Guy's Hospital Medical School, London SE1 9RT, U.K.

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SUMMARY

Murine cytomegalovirus (MCMV) may be rapidly and reproducibly attenuated by passage in tissue culture. A high proportion of CD1 mice neonatally infected with the virulent strain of CMV can be shown to have replicating virus in the kidneys for up to 90 weeks p.i. and this high rate of isolation is not altered in pregnancy. A much lower rate of isolation from the kidneys is seen in CD1 mice neonatally infected with the attenuated strain. However, this isolation rate was significantly increased during pregnancy and higher titres of virus were recovered.

INTRODUCTION

Human cytomegalovirus (CMV) has been of great clinical interest because of its ability to be reactivated in the immunosuppressed and transfused patient (Lang, 1972). Reports from Olding et al. (1975) and Jordan et al. (1977) have recorded experimental reactivation of murine CMV in infected mice. Reactivation was induced by co-cultivation of lymphocytes in vitro with histo-incompatible cells, or by immunosuppression of chronically infected mice by anti-lymphocyte serum or corticosteroids.

In humans there is an increase in the rate of CMV excretion during pregnancy. Virus is recovered from the cervix of up to 13.4% of pregnant females as compared to an isolation rate of 1% in the general population (Reynolds et al. 1973). No animal model has been published previously for study of such reactivation and this report describes an investigation of the effects of pregnancy on mice infected with CMV up to 70 weeks previously.

METHODS

Mice. SPF Charles Rivers CD1 outbred mice were used throughout this study. Primary mouse embryo fibroblasts (MEF) were prepared by the trypsinization of foetuses from 18 day-old pregnant CD1 females. Cells were grown in Eagle's MEM supplemented with 5% foetal calf serum (FCS) and 0.11% NaHCO₃, and were not subcultured beyond the third passage, at which point susceptibility to MCMV began to wane (J. J. Gould, unpublished data).

Virus. The Smith strain of virus was kindly supplied by Dr J. Osborne, Department of Microbiology, University of Wisconsin, U.S.A. It had been isolated from a colony of chronically infected mice in 1954 and had been grown exclusively in vivo from that date. The strain was coded OSG and was propagated in vivo by the intraperitoneal (i.p.) inoculation of 3 week-old mice with approx. 10⁶ p.f.u. of virus. Three weeks later the mice were sacrificed, the salivary glands pooled and homogenized in a small volume of Eagle's MEM with 0.11% NaHCO₃, then ultrasonicated briefly and clarified by low speed centrifugation. The virus pool was stabilized with an equal volume of 40% sorbitol and stored at −70°C.

On receipt the OSG strain was passaged eight times in MEF cultures and this attenuated strain, as judged on mouse lethality tests, was designated OTC 8. It was harvested as fluid and cells from infected MEF cultures. The virus pool was ultrasonicated, clarified by low speed centrifugation, stabilized with an equal volume of 40% sorbitol and stored at −70°C.
Organs for virus assay were removed aseptically, homogenized, ultrasonicated and clarified by low speed centrifugation. The supernatant was titrated by the plaque assay method. Samples of urine were collected by syringe from the bladder and assayed in a similar manner.

**Assay methods.** Tissues for histological examination were either snap-frozen in liquid nitrogen for cryostat sectioning, or fixed in formal saline for routine histology. Frozen sections were fixed in acetone and stained for CMV antigens by the indirect immunofluorescence method (Mims & Gould, 1978). Serum antibody titrations were carried out by the indirect immunofluorescence method on slide cultures of MEF infected with OTC 8 virus (Mims & Gould, 1978).

Infectivity assays were made by adding dilutions of test material in Eagle's MEM supplemented with 5% FCS to monolayers of MEF cells in Falcon multiwell dishes. After 60 min adsorption at 36-5 °C, the cultures were overlaid with Eagle's MEM containing 0.088% NaHCO₃, 2% FCS and 0.7% carboxymethylcellulose. The overlay was tipped off 4 days after infection, the cultures stained and plaques counted on a Zeiss-Jena Dokumator overhead microscope.

**Design of experiment.** Neonatal mice received 10³ p.f.u. of OSG or OTC 8 by the i.p. route and when 21 days old were weaned and caged separately according to sex. At different times females were mated with uninfected males, and at 16 to 18 days gestation the females were sacrificed and samples collected. Non-mated females and males were examined in parallel.

**RESULTS AND DISCUSSION**

During the first 2 weeks of life, virus was isolated from the kidney and salivary glands of the majority of mice receiving either virulent (OSG) or attenuated (OTC 8) virus. Mice surviving infection with virulent virus showed a high rate of isolation from the salivary glands at 3 to 10 weeks (Table 1) but after this there was a reduction in the number of isolations and none was made after 50 weeks. Similarly, many positive kidneys were found during the first 50 weeks and again there was an apparent fall off at later times. Mice receiving attenuated virus showed an equally high rate of isolation from the salivary glands in the first 10 weeks of life, after which isolations were rare. Virus was frequently found in the kidneys of neonatally infected mice during the first 2 weeks of life (Mims & Gould, 1978), but by 3 to 10 weeks virus was not isolated in this study. Between 10 and 110 weeks, however, the isolation rate varied from 25 to 50%.

The other tissues tested were liver, spleen, adrenals, heart, lungs, pancreas, brain, genital tract and urine. Virus was not recovered from these sites except during the first 2 weeks and urine was at all times negative. The conceptus was similarly negative in pregnant mice.

Mice infected up to 70 weeks previously were mated and sacrificed at 16 to 18 days gestation. Isolation rates from those infected with the virulent virus strain were similar to those in the previous experiment and neither rates nor mean titres differed in pregnant and non-pregnant mice (Table 2). With the attenuated strain the isolation rate was significantly increased \( (P < 0.01) \) in pregnant mice and the mean kidney titre was also higher \( (P < 0.01) \). One salivary gland was positive in the group of pregnant mice infected with virulent virus, but all materials tested from other mice were negative.

Routine histological and immunofluorescent examination was made of kidneys and salivary glands from pregnant and non-pregnant mice from which virus had been isolated, but foci of infection could not be located. CMV antibody titres did not differ between pregnant and non-pregnant mice, and remained unchanged throughout pregnancy.

The results obtained with virulent virus are in accordance with the early work of Medearis (1964) who also showed that the rate of isolation from a chronic infection established by
Reactivation of cytomegalovirus

Table 1. Isolation of infectious virus from the kidneys and salivary glands of mice given $10^8$ p.f.u. of virulent or attenuated virus by the i.p. route in the first 24 h of life

<table>
<thead>
<tr>
<th>Time p.i. (weeks)</th>
<th>Positive isolations/total mice tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virulent Kidney</td>
</tr>
<tr>
<td>3-10</td>
<td>2/7</td>
</tr>
<tr>
<td>10-30</td>
<td>9/13</td>
</tr>
<tr>
<td>30-50</td>
<td>7/9</td>
</tr>
<tr>
<td>50-70</td>
<td>0/5</td>
</tr>
<tr>
<td>70-90</td>
<td>2/6</td>
</tr>
<tr>
<td>90-110</td>
<td>ND*</td>
</tr>
</tbody>
</table>

* ND, Not done.

Table 2. Isolation of infectious virus from the kidneys* of pregnant and non-pregnant mice infected i.p. with virulent or attenuated virus during the first 24 h of life

<table>
<thead>
<tr>
<th>Time p.i. (weeks)</th>
<th>Positive isolations</th>
<th>Mean titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virulent Non-pregnant</td>
<td>9/13</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>8/11†</td>
</tr>
<tr>
<td></td>
<td>Attenuated Non-pregnant</td>
<td>8/29</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>7/14</td>
</tr>
</tbody>
</table>

* Kidneys were titrated individually and the mean titre expressed as p.f.u./g tissue.
† One positive salivary gland taken from mouse 22 weeks p.i.

neonatal inoculation was not altered in pregnancy. Until now a similar study had not been carried out with attenuated virus. With attenuated virus there was a significant (twofold) increase in isolation rate from kidneys during pregnancy and a significant (eightfold) increase in mean titre. Many organs and tissues are infected during the first few weeks after neonatal infection, but thereafter, only kidneys or salivary glands contain infectious virus (Mims & Gould, 1978). The products of conception remain negative during pregnancy and antigen-containing cells are not detected on immunofluorescence examination. These experiments gave little information about the nature of the infection in mice whose kidneys became positive during pregnancy. Conceivably there was a low grade chronic infection below the level of detectability, i.e. less than 50 p.f.u./g tissue; alternatively, virus DNA was present in cells with little or no production of antigen or infectious virus.

The source of reactivating virus could be lymphocytes, a focus of latency identified by Olding et al. (1975) but if so, it is difficult to account for its confinement to the kidney. Although high levels of antibody might theoretically limit the spread of virus from the kidney, this has not always been shown to be true for human CMV (Lang, 1972). It is possible that the focus of latent infection is in tubular epithelial cells in the kidney. Activation could be attributed to immunosuppression occurring naturally during the course of pregnancy, or alternatively to the hormonal changes of pregnancy. Experiments are in progress to distinguish between these alternatives.

REFERENCES


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