Leukaemia Reactivates Mouse Cytomegalovirus

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SUMMARY

An established model for latency by mouse cytomegalovirus (MCMV) was used to assess the effects of leukaemia on reactivation of MCMV. After inoculation of 400 spleen focus-forming units of Friend leukaemia virus (FLV), a significant number of mice yielded MCMV in salivary glands and kidneys 4 to 5 weeks later. Administration of cyclophosphamide during FLV infection increased the percentage of MCMV-positive animals. These results suggest that cytomegalovirus infections in leukaemic patients may reactivate as a result of the leukaemia itself and may be exacerbated by chemotherapy.

Cytomegalovirus (CMV) can persist within the human host in an undetectable form for many years after primary infection. These inapparent or latent CMV infections can reactivate and result in a wide range of clinical manifestations, ranging from asymptomatic shedding of virus to severe fulminant infection and even death. Immunocompromised patients, organ transplant recipients, victims of neoplasia and patients undergoing chemotherapy are at increased risk of contracting active CMV infections. The mechanisms responsible for the reactivation of these infections are generally unclear because of co-existing disease states and various therapeutic modalities. However, immunological stimuli, chemotherapy and transfer of infection within transplanted tissues or blood components have all been implicated as causative mechanisms (Betts & Hanshaw, 1977; Ho, 1977).

Research on latency using various murine systems increased after it was reported that mouse spleen cells could harbour mouse CMV (MCMV) in a latent form (Olding et al. 1975). Immunosuppression in vivo induced by cyclophosphamide (CY) (Mayo et al. 1977) or by anti-lymphocyte serum and corticosteroids (Jordan et al. 1977) has been shown to reactivate latent MCMV; transfer of latently infected blood cells (Cheung & Lang, 1977) and spleen cells (Mayo et al. 1978) yielded similar results. Co-cultivation of spleen cells under either strictly allogeneic conditions (Olding et al. 1975) or both allogeneic or syngeneic conditions (Mayo et al. 1978; Wise et al. 1979) reactivates MCMV in vitro. Although differences exist among models, these findings demonstrate that, depending upon the circumstances, one or more mechanisms may interact to cause CMV infections. We have continued to use one of these mouse models to determine whether leukaemia, an immunocompromising disease frequently associated with CMV infection, can cause active MCMV infections in a latently infected host.

MCMV strain Smith (originally supplied by Dr June Osborn, University of Wisconsin Medical School, Madison, Wis., U.S.A.) has been maintained by passage of 10% (w/v) salivary gland homogenates in CD-1 mice (Charles Rivers Laboratories, Wilmington, Mass., U.S.A.). Organ homogenates (10%, w/v) were assayed for the presence of MCMV in secondary mouse embryo cells (MEC) prepared from 12- to 14-day-old CD-1 embryos (Charles Rivers Laboratories) by procedures described previously (Mayo et al. 1977). The Eckner NB tropic strain (Eckner, 1973) of Friend leukaemia virus (FLV) was prepared in DBA/2 mice (Jackson Laboratories, Bar Harbor, Me., U.S.A.). Clarified homogenates of infected spleens were prepared 10 days after intraperitoneal (i.p.) injection of stock FLV and frozen at −70 °C.
Table 1.  Proliferative response of normal and FLV-infected spleen cells to Con A

<table>
<thead>
<tr>
<th>Time after FLV infection (days)</th>
<th>Spleen cells</th>
<th>$[^{3}H]$-TdR incorporated (ct/min)*</th>
<th>Increment of incorporation†</th>
<th>Transformation index‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Normal</td>
<td>27435</td>
<td>26898</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>FLV-infected</td>
<td>15347</td>
<td>14510</td>
<td>0.54</td>
</tr>
<tr>
<td>4</td>
<td>Normal</td>
<td>10147</td>
<td>9588</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>FLV-infected</td>
<td>7267</td>
<td>6633</td>
<td>0.69</td>
</tr>
</tbody>
</table>

* Each value in ct/min represents the arithmetic mean of triplicate cultures.  
† Increment of incorporation = (mean ct/min Con A-stimulated cultures) minus (mean ct/min unstimulated cultures).  
‡ Transformation index = FLV-infected increment of incorporation / Normal increment of incorporation.

Five- to six-week-old DBA/2 mice (Jackson Laboratories) were infected i.p. with $2 \times 10^5$ p.f.u. of MCMV and housed in special isolation chambers. Approx. 5 months after primary MCMV infection, virus was not detectable by standard plaque assay of organ homogenates. These animals were then operationally defined as latently infected (Mayo et al. 1977). FLV infection was initiated in latently infected mice by an i.p. injection of 400 spleen focus-forming units of the FLV complex. Cyclophosphamide (Cytoxan Mead Johnson Laboratories, Ind., U.S.A.) was administered in 150 mg/kg doses.

The degree of lymphocyte stimulation was measured by incorporation of $[^{3}H]$-thymidine ($[^{3}H]$-TdR) into acid-insoluble material after concanavalin A (Con A) stimulation of splenic leukocytes. Cultures were prepared by pooling two spleens for each time point examined. Spleens were minced into fine pieces and then pressed through a stainless steel mesh. Red blood cells were removed by a 10 min hypotonic saline treatment. After equilibration with hypertonic saline, cell suspensions were pelleted, resuspended in RPMI medium and then counted. Cells ($2 \times 10^8$) were added to sterile Wassermann tubes containing 1 ml RPMI medium and 2 μg Con A where appropriate. After incubation at 37 °C for 3 days, 1 μl $[^{3}H]$-TdR (sp. act. 52.2 Ci/mmol) was added to each culture tube which was incubated for an additional 4 h. Cultures were then centrifuged, and the resulting cell pellets were treated with a 0.1 ml solution of 1 mg Pronase/ml of 1 % Sarcosyl for 15 min. Cell suspensions were then passed through 25-gauge syringe needles and 100 μl was spotted on to filter paper discs. The discs were washed consecutively in trichloroacetic acid, 95 % alcohol, acetone and then dried. The amount of $[^{3}H]$-TdR incorporation was determined in a Beckman LS 9000 liquid scintillation counter.

Leukaemia victims suffer increased morbidity and mortality rates due to CMV infections (Levine et al. 1974; Abdallah et al. 1976). The CMV source, whether exogenous or reactivated from a previous infection, is usually unknown. Immunosuppressive treatments, such as those received by leukaemia patients, have already been shown to reactivate MCMV in mouse models. The question remained as to whether the debilitating effects of leukaemia, aside from the accompanying chemotherapy, were sufficient to cause reappearance of infectious virus in a latently infected host.

We approached this problem by first confirming the immunosuppressive effects of FLV in our system. DBA/2 mice are homozygous for the dominant Fv-2* allele controlling restriction to FLV disease and are, therefore, fully susceptible to FLV (Lilly & Pincus, 1973). One advantage of the FLV system is the absence of a latent period between injection of the virus and effect on the host. Using a lymphoblast transformation assay, decreased $[^{3}H]$-TdR uptake of Con A-stimulated spleen cells from FLV-infected animals was readily demon-
Table 2. Effect of FLV and CY on reactivation of MCMV in latently infected DBA/2 mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Salivary gland</th>
<th>Kidney</th>
<th>Liver</th>
<th>Lung</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0/16* (0%)</td>
<td>1/16</td>
<td>0/16</td>
<td>0/16</td>
<td>0/16</td>
</tr>
<tr>
<td>FLV</td>
<td>5/31 (16%)</td>
<td>15/31</td>
<td>0/31</td>
<td>0/31</td>
<td>0/31</td>
</tr>
<tr>
<td>FLV+CY</td>
<td>4/11 (36%)</td>
<td>6/11</td>
<td>0/11</td>
<td>0/11</td>
<td>0/11</td>
</tr>
</tbody>
</table>

* Number of animals with MCMV total.
† All probabilities based on Chi-square evaluation compared to no treatment group.

strated 2 days after FLV infection (Table 1). A similar level of unresponsiveness was found 4 days after infection (Table 1) which remains below normal for the life of the animal (Ceglowski et al. 1974). Four to 5 weeks after FLV infection, organs were removed and their homogenates (10%, w/v) titrated on MEC to detect MCMV. At this time, all FLV-infected animals had considerable splenomegaly, a hallmark of FLV infection; spleen weights varied from 10 to 15 times those of normal spleens. Splenic foci were too numerous to count using an assay for spleen focus-forming virus (Axelrad & Steeves, 1964).

The results of organ titrations are shown in Table 2. In the untreated control group only one animal had detectable infectious virus, which most probably represents spontaneous reactivation, a phenomenon noted in other mouse models (Jordan et al. 1977). In animals receiving FLV only, 16% (5 of 31) had infectious virus in salivary glands, whereas 48% (15 of 31) had infectious virus in kidneys. Treatment of FLV-infected animals with two injections of CY (one during the third and one during the fourth week after FLV infection) increased the percentage of MCMV-positive salivary glands (36%; 4 of 11) and slightly increased the percentage of virus-positive kidneys (55%; 6 of 11). MCMV was not detected in liver, lung or spleen.

The increase in the percentage of MCMV isolations from the salivary glands of FLV plus CY-treated animals was anticipated since previous experience with this model has demonstrated reactivation of MCMV almost exclusively in the salivary glands of CY-treated mice (Mayo et al. 1978). The occurrence of a large number of virus-positive kidneys in both the FLV and FLV plus CY-treated groups, however, was not expected. These findings are similar to those of Jordan et al. (1977) who reported isolation of MCMV from salivary gland, kidney, liver, lung and spleen after anti-lymphocyte serum and cortisone treatment of latently infected C3H mice. Recently, we have noted that immunosuppressive effects of FLV increase the spread of MCMV throughout the animal during acute MCMV infections (Mayo & Rapp, 1980). The dissemination of MCMV may, therefore, be expected to vary depending on the animal model and regimen of immunosuppressive agents used.

The reactivation of MCMV by leukaemia is another means that can be used to experimentally demonstrate virus latency (Hudson et al. 1979). This finding also points out another biological similarity between MCMV and human CMV (Hudson et al. 1979; Mayo & Rapp, 1980). The increased morbidity and mortality of leukaemia patients due to CMV (Levine et al. 1974; Abdallah et al. 1976) may be caused, in part, by reactivation of the virus within the host by the leukaemia itself, as this report suggests. The added burden placed on the host by anti-leukaemia chemotherapy further enhances the chance of disease due to reactivated CMV.

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REFERENCES


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