Replication of lactate dehydrogenase-elevating virus in cells infected with murine leukaemia viruses in vitro

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Although the majority of mouse strains infected with lactate dehydrogenase-elevating virus (LDV) do not show any particular symptoms, the virus is able to induce acute poliomyelitis in C58 or AKR mice. Murine leukaemia virus (MuLV) has been detected at a high titre in the spinal cord of affected mice. In this study, we have analysed the possible role of MuLV in the induction of neurological disease by LDV. Immunofluorescent staining, autoradiography and an infectivity assay of virus yield have shown that LDV replicated in continuous mouse and rat cell lines that had been infected with an ecotropic MuLV isolated from C58 mice, but did not replicate in cells not infected with MuLV. No significant differences in infection were observed among the various ecotropic MuLVs employed, except for Friend leukaemia virus which rendered the cells susceptible to LDV least efficiently. The infectivity of the neurovirulent strain, LDV-C, to MuLV-infected cells was 50- to 100-fold greater than that of the avirulent strains (LDV-N, -Nu, -R and -P). The infectivity to macrophages was almost the same for virulent and avirulent strains. Adsorption studies using a radiolabelled virus revealed that LDV-C was adsorbed to MuLV-infected cells more efficiently than the avirulent strain, LDV-N. The difference in infectivity to these cells, therefore, may be due in part to the difference in adsorption rate. This may suggest differences in the interaction of the viral proteins with MuLV-infected cells from those with macrophages at the initiation of virus infection. These results may be relevant to the mechanisms of paralytic disease caused by LDV infection in C58 mice.

Introduction

Lactate dehydrogenase-elevating virus (LDV) induces life-long persistent viraemia in ordinary mice; however, no significant symptoms have been reported except for an elevation of certain plasma enzymes and deposition of immune complexes in the kidneys (Riley et al., 1960; Oldstone & Dixon, 1971; Rowson & Mahy, 1975). Murphy et al. (1970) described a polioencephalomyelitis in C58 mice receiving transplanted syngeneic tumour cells. They thought that the disease was induced by an autoimmune mechanism due to presumed antigens common to the tumour cells and the central nervous system (CNS). Martinez et al. (1979) and Nawrocki et al. (1980), however, identified LDV as the aetiologic agent, which was present in tumour cells from transplanted mice. LDV has been shown to replicate in motor neurons in the anterior horn of the spinal cord, resulting in destruction of the cells and paralysis (Kascak et al., 1983; Stroop & Brinton, 1983; Stroop et al., 1985; Contag et al., 1986).

Pease demonstrated that strains of mice susceptible to the paralytic disease are the leukaemia-prone C58 and AKR lines and that the incidence of paralysis is restricted by the Fv-1 locus (Pease & Murphy, 1980; Pease et al., 1982). The Fv-1 gene regulates the expression and replication of endogenous murine leukaemia virus(es) (MuLV) in the host (Rowe et al., 1973). The susceptibility of mice to the disease increased with age and immune suppressive treatments (Duffey et al., 1976; Martinez et al., 1980).

The target cells for LDV are macrophages, and no permissive cells or cell lines other than macrophages have yet been found (Stueckemann et al., 1982). Whereas in the spinal cord of paralysed mice, LDV was shown to infect and replicate in motor neurons and other CNS-specific cells, no replication has been observed in the CNS of disease-resistant mice (Kascak et al., 1983; Stroop & Brinton, 1983).

Retroviruses integrate their proviral DNA into a host cell chromosome during the replication cycle and in general multiply without inducing cell destruction. MuLV replicates in several cell lines of mouse and some other species. In this paper, we have investigated whether LDV could infect and replicate in MuLV-infected cells, in an attempt to elucidate the role of MuLV in the induction of paralytic disease caused by LDV.
Methods

Mice. For the preparation of stock virus and peritoneal macrophages, 4-week-old, male, outbred ddY mice were used. Eight-week-old, female BALB/c mice were used for monoclonal antibody (MAb) preparation. All mice were purchased from the Shizuoka Animal Breeding Center. C58/J mice, aged 3 months, were donated by Dr H. Yoshikura, Tokyo University and the rat cell line NRK were used for MuLV infection and propagation. Cells were passaged and maintained with MEM plus 10% foetal calf serum (FCS). Breeding Center. C58/J mice, aged 3 months, were donated by the XC plaque procedure (Rowe et al., 1975). The spleens were homogenized in MEM plus 10% FCS, centrifuged and the supernatants were inoculated into NIH/3T3 cells. The cells were passaged every 3 days and the culture media were collected 1 to 2 weeks later. These media were used as virus preparations. Other ecotropic MuLVs employed were FN2N7 (Friend leukaemia virus helper, N-tropic), MoIoney leukaemia virus (NB-tropic) and WNBN7 (B-tropic). They were kindly donated by Dr Yoshikura. Titres of the viruses were 10^6 to 10^7 p.f.u. per ml as assayed by the XC plaque procedure (Rowe et al., 1970).

Monoclonal anti-LDV antibody and anti-MuLV serum. The source of spleens for the preparation of the MAb was BALB/c mice infected intraperitoneally (i.p.) 2 to 3 months before with LDV-N. The spleens were removed from mice aseptically and a single cell suspension was made. The spleen cells were fused with myeloma cells (SP2/0-Ag14) and specific antibody-producing cells were screened by immunofluorescence (IF) staining (Köhler & Milstein, 1975). The hybridoma supernatant (immunoglobulin content approximately 50 μg/ml) was used as the MAb. From several MABs obtained, clone no. 8 (IgG2a) was used for IF staining, because of its strong reactivity with infected cells. The specificity of the clone is anti-LDV VP1 (unpublished results). A MAb to human parvovirus B19 with the same isotype as clone no. 8 was used as the control. Rabbit anti-MuLV (Friend virus) serum was kindly donated by Dr Yoshikura (Yokota et al., 1984). The serum cross-reacted with all MuLVs employed in this experiment as assessed by IF staining.

Infection of LDV in MuLV-infected cells and IF staining. The cells described above were incubated on a slide (10 wells; Bokusui Brown) at 37 °C in 5% CO2 in air. Each well was seeded with cells at about 10^4 cells/well. The cells were then infected with MuLV at an m.o.i. of 0.1 to 1.0 p.f.u. per cell in medium containing polybrene (10 μg/ml; Sigma). After 7 to 10 days, the cells were infected with LDV at an m.o.i. of 1000 ID50/cell. Ten hours later, the cells were dried, fixed with acetone and stained with the anti-LDV MAb (dilution 1/1000) and fluorescein isothiocyanate (FITC)-labelled goat anti-mouse IgG (Cappel Laboratories) as described earlier (Inada & Mims, 1984). For double staining, the cells were incubated with the anti-MuLV serum (dilution 1/300) plus anti-LDV followed by FITC-labelled anti-mouse IgG plus rhodamine isothiocyanate (RTTC)-labelled-goat anti-rabbit IgG (Cappel Laboratories). The percentage of antigen-positive cells was calculated after counting 5000 to 10000 cells on each culture.

For the titration of virus yield, LDV-infected cells cultured in 24-well plates were frozen and thawed three times, centrifuged and the infectivity of LDV in the supernatant was assayed.

Microautoradiography. This was done by essentially the same method as reported previously (Tong et al., 1977). Peritoneal macrophages or MuLV-infected D3hIg cells cultured on slides were infected with LDV at an m.o.i. of about 1000 ID50/cell. Actinomycin D (Sigma, 1 μg/ml) was added 5 h later followed by incubation for 2 h at 37 °C. Then [3H]uridine (New England Nuclear; 20 μCi/ml) was added to label the viral RNA for 2 h at 37 °C. The cells were dried, fixed, and washed with 10% TCA and coated with emulsion (Sakura NRM2) for autoradiography.

Preparation of radiolabelled LDV. Peritoneal macrophages were obtained from ddY mice and incubated in plastic bottles (75 cm²) for 3 h. Non-adherent cells were removed by washing with MEM and the adherent macrophages were infected with LDV at an m.o.i. of 1000 ID50/cell. After 5 h, [3H]uridine (NEN) was added at 10 μCi/ml and the cells were incubated for additional 13 h at 37 °C in 5% CO2 in air. The supernatants together with the cells were collected, frozen and thawed once and centrifuged at 5000 r.p.m. for 10 min. The labelled virus in the supernatant was purified as previously described (Brinton-Darnell & Plagemann, 1975).

Adsorption of radiolabelled virus to MuLV-infected cells. [3H]-labelled LDV (approx. 500 000 c.p.m./0.2 ml) was added to MuLV-infected or uninfected SC-1 cells which had been cultured on a 24-well plastic plate (Costar). Virus adsorption was done at 4 °C for 30 min with frequent agitation (every 5 min). The cells were then washed five times with cold PBS and RNA was extracted by the hot SDS–phenol (60 °C) procedure. The RNA was precipitated with TCA, collected by filters and the radioactivity was determined using a scintillation counter.

Results

Replication of LDV in cells infected with MuLV

No cell or cell line, other than macrophages, that is permissive for LDV in vitro has yet been found. LDV did not replicate in NIH/3T3, BALB/3T3, SC-1, D3hIg or NRK cells (Table 1, Fig. 1 and 2). However, when these cells were infected with ecotropic MuLV isolated from C58 mice, LDV antigen-positive cells were seen after infection with LDV-C. At a maximum, approximately 50% of the MuLV-infected SC-1 cells were stained with the anti-LDV VP1 MAb. The control antibody did not detect the LDV antigen. The percentage of LDV-infected cells was lower in MuLV-infected NIH/3T3, D3hIg or NRK cells, although the same conditions of MuLV infection had been used (Table 1). When the input amount of MuLV was decreased, so that the infection would become focal, the LDV-infected cells were restricted to a MuLV-infected area as shown by double staining using the anti-MuLV antibody and anti-LDV MAb (Fig. 3).
Table 1. Replication of LDV in cells infected with C58-MuLV*

<table>
<thead>
<tr>
<th>Cells</th>
<th>NIH/3T3</th>
<th>BALB/3T3</th>
<th>SC-1</th>
<th>D3hlg</th>
<th>NRK</th>
</tr>
</thead>
<tbody>
<tr>
<td>MuLV-uninfected</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>MuLV-infected</td>
<td>1.2 ± 0.5† (&gt; 90)§</td>
<td>0.7 ± 0.4 (approx. 20)</td>
<td>46.1 ± 6.9 (&gt; 90)</td>
<td>4.8 ± 0.8 (&gt; 90)</td>
<td>0.03 ± 0.02 (approx. 30)</td>
</tr>
</tbody>
</table>

* Mouse and rat cell lines infected with C58-MuLV were infected with LDV-C at a multiplicity of 1000 ID₅₀/cell 10 days after MuLV inoculation. The cells were infected with MuLV at an m.o.i. of 1.0 p.f.u./cell. Fluorescence-positive cells were counted after staining with anti-LDV MAb.
† ---, Not detectable.
§ Each figure represents the mean ± s.d. calculated from counts on three to five cultures.

LDV replication in MuLV-infected cells

MuLV-infected D3hlg cells were labelled with [³H]uridine and autoradiography was done to see whether LDV RNA would be synthesized after infection. In parallel, peritoneal macrophages cultured in vitro were infected with LDV and autoradiograms were made for comparison. There were about 10% grain-positive cells in the LDV-infected macrophage culture (photograph not shown) and as shown in Fig. 4, a similar number of grain-positive cells was observed in LDV-infected, MuLV-infected cells.

Fig. 1. IF staining of LDV and MuLV antigens. C58-MuLV-infected (a), uninfected (b) SC-1 cells stained with anti-LDV VP1 MAb and FITC-goat anti-mouse IgG, and C58-MuLV-infected (c) and uninfected (d) SC-1 cells by anti-MuLV rabbit serum and RITC-goat anti-rabbit IgG. Bar marker represents 50 μm.
Fig. 2. LDV replication in SC-1 cells infected with MuLV. SC-1 cells cultured in a 24-well plate were infected with C58-MuLV at an m.o.i. of 1·0 p.f.u./cell. Ten days later, the cells were infected with LDV-C at an m.o.i. of 1000 ID₅₀/cell for 4 h and residual virus was removed by washing with MEM. The cells were frozen and thawed three times, centrifuged and the infectivity for LDV in the supernatant assayed as described in Methods. (O) MuLV-infected and (●) uninfected cells.

The supernatants of disrupted MuLV-infected cells were obtained after LDV infection and their infectivity for LDV was assayed. The titre began to increase from 6 h post-infection (p.i.) and peaked at about 12 h p.i. (Fig. 2). At 48 to 96 h p.i., an apparent c.p.e. with cell rounding and atrophy was observed in LDV-infected, C58-MuLV-infected SC-1 cultures.

LDV replication in cells infected with MuLV before or after LDV inoculation

As LDV has been demonstrated to infect and replicate in cells infected 7 to 10 days before with MuLV, we investigated whether LDV can also replicate in cells infected with MuLV after LDV inoculation. SC-1 cells were inoculated with LDV and, at the same time or at appropriate times later, with MuLV. LDV-infected cells were not observed in cultures infected with MuLV 1 to 10 days after LDV inoculation. There were only a few LDV-infected cells in cultures infected with LDV and MuLV simultaneously (Table 2). It is therefore evident that LDV can replicate only in cells which were already infected with MuLV.

Replication of LDV in mouse cell lines infected with various MuLV strains

We next investigated whether cells, when infected with different MuLV strains, become permissive for LDV. NIH/3T3, BALB/3T3, SC-1 and D3hlg cells were infected with N-tropic Friend, NB-tropic Moloney and B-tropic MuLV. Seven to 10 days later, the cells were infected with LDV-C and IF-positive cells were counted. Table 3 shows that LDV could infect and replicate in the cells infected with each of the MuLVs, although its infectivity in Friend virus-infected cells was considerably lower. Friend MuLV, however, replicated to the same degree as the other MuLV strains in the cell lines examined (data not shown).

Infectivity of various LDV strains to MuLV-infected cells

The LDV-C strain, isolated from a C58 mouse tumour, is neurovirulent and the strains N, Nu, R and P are avirulent (Martinez et al., 1980). These strains were
There were no significant differences among these avirulent strains.

The target cells for LDV in vivo are macrophages and about 10% of resident peritoneal macrophages are susceptible to infection in vitro (Stueckemann et al., 1982; Inada & Mims, 1984). We next compared the infectivity of LDV strains to macrophages. Table 4 shows there were no differences in the percentage of infected cells, nor of infectivity in the culture supernatant among those strains. These results indicate that whereas infectivity to macrophages was not significantly different between the virulent and avirulent strains, the virulent LDV-C showed a 50 to 100-fold higher infectivity to MuLV-infected cells than the avirulent, LDV-N, -Nu, -R and -P strains.

Differential adsorption rates of neurovirulent and avirulent strains of LDV to MuLV-infected cells

We have observed that some MAbs to the envelope glycoprotein VP3 of LDV discriminate between the virulent and avirulent strains (unpublished results). Although the function of VP3 has not yet been elucidated, it may be related to adsorption, penetration or uncoating, i.e. an early stage of the virus growth cycle. Therefore we examined whether the difference of infectivity to MuLV-infected cells was due to a difference in virus adsorption. LDV strains were radiolabelled in vitro with [3H]uridine, purified and adsorbed to C58-MuLV-infected or uninfected SC-1 cells. Whereas avirulent virus was adsorbed to MuLV-infected cells at the same rate as to uninfected cells, virulent LDV-C was adsorbed to MuLV-infected cells approximately three times more than to uninfected cells (Table 5). These results indicate that the difference of infectivity between virulent and avirulent strains may be due in part to the difference in their adsorption to MuLV-infected cells.

Table 2. No replication of LDV in cells infected with MuLV after LDV inoculation

<table>
<thead>
<tr>
<th>MuLV infection*</th>
<th>-10†</th>
<th>-5</th>
<th>-3</th>
<th>-1</th>
<th>0</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MuLV-uninfected SC-1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>MuLV-infected SC-1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0.01 ± 0.01§</td>
<td>12.7 ± 1.7</td>
<td></td>
</tr>
</tbody>
</table>

* SC-1 cells were inoculated with LDV-C and at appropriate times infected with C58-MuLV as in Table 1. The cells were stained 24 h later with anti-LDV MAb.
† Days of LDV inoculation after MuLV infection.
‡ —, Not detectable.
§ Each figure represents the mean ± s.d. calculated from counts on three to five cultures.
Table 3. LDV replication in cells infected with various MuLV strains*

<table>
<thead>
<tr>
<th>MuLV</th>
<th>LDV antigen-positive (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIH/3T3 (Fv-l⁺)</td>
<td>BALB/3T3 (Fv-l⁺)</td>
</tr>
<tr>
<td>N-tropic Friend</td>
<td>&lt;0.1† (&gt;90)‡</td>
<td>ND§</td>
</tr>
<tr>
<td>NB-tropic Moloney</td>
<td>ND</td>
<td>0.5–1 (approx. 80)</td>
</tr>
<tr>
<td>B-tropic MuLV</td>
<td>ND</td>
<td>5–20 (&gt;90)</td>
</tr>
</tbody>
</table>

* Mouse cell lines were infected with the various MuLV strains indicated at an m.o.i. of 0.1 to 1.0 p.f.u./cell. Seven to 10 days later, the cells were infected with LDV and stained with anti-LDV MAb or anti-MuLV serum as in Table 1.
† Each figure indicates the range of percentages of positive cells derived from the counts of three to five experiments.
‡ The figures in parentheses indicate percentage of positive cells of MuLV-infected cells.
§ ND, Not done.

Table 4. Differences in the ability of LDV to replicate in MuLV-infected cells*

<table>
<thead>
<tr>
<th>Cells</th>
<th>LDV-N</th>
<th>LDV-C</th>
<th>LDV-Nu</th>
<th>LDV-R</th>
<th>LDV-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>C58-MuLV-infected</td>
<td>0.5–1</td>
<td>1–5</td>
<td>0.5–1</td>
<td>0.5–1</td>
<td>0.5–1</td>
</tr>
<tr>
<td>SC-1</td>
<td>10⁷‡</td>
<td>10⁷</td>
<td>10⁸</td>
<td>10⁸</td>
<td>10⁷</td>
</tr>
</tbody>
</table>

* C58-MuLV-infected SC-1 or macrophages cultures in vitro were infected with neurovirulent (LDV-C) and avirulent (LDV-N, -Nu, -R, -P) strains at an m.o.i. of 1000 ID₅₀/cell.
† Numbers of MuLV-infected SC-1 cells and macrophages were 5 × 10⁴ and 10⁴ per well in microscope IF slide, 5 × 10⁴ and 10⁵ per well in 24 wells, respectively.
‡ Each figure represents the mean of infectivity of two wells.

Table 5. Adsorption of radiolabelled LDV to MuLV-infected cells*

<table>
<thead>
<tr>
<th>Cells</th>
<th>Adsorbed virus (c.p.m.)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDV-N</td>
<td>LDV-C</td>
<td></td>
</tr>
<tr>
<td>C58-MuLV-uninfected</td>
<td>59.5 + 8.3‡</td>
<td>47.5 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>C58-MuLV-infected</td>
<td>50.8 ± 7.5</td>
<td>173.3 ± 34.9‡</td>
<td></td>
</tr>
</tbody>
</table>

* [³H]Uridine-labelled purified LDV-N and -C (approx. 500 c.p.m.) were adsorbed to C58-MuLV-infected cells at 4 °C for 30 min. The cells were washed thoroughly with PBS, RNA was extracted and their radioactivity was counted.
† Each figure represents the mean ± SD from four wells.
‡ Significantly greater than c.p.m. of LDV-C in MuLV-uninfected cells (P < 0.001) by Student’s t-test.

Discussion

The target cells for LDV replication in vivo are macrophages. Cells or cell lines other than macrophages which are permissive for LDV in vitro have not yet been detected despite extensive study (Stueckemann et al., 1982; Rowson & Mahy, 1985). The cells employed in this study also failed to support LDV infection and replication unless these cells had been infected with ecotropic MuLV. Infection by LDV was observed in various ecotropic MuLV-infected cells, but the extent of infection varied considerably from virus strain to strain and from cell line to cell line. To render the cells susceptible to LDV replication, they had to be infected with MuLV before LDV inoculation. As the titre of LDV in the culture supernatant was low at early stages of infection, the supernatants of disrupted MuLV-infected culture were obtained after LDV infection for titration. A significant increase in virus yield was seen in the MuLV-infected culture.

In the double staining experiment, we observed some cells in which the LDV but not the MuLV antigen was positive. The reasons for this are unknown but the synthesis of MuLV antigens may have decreased due to rapid replication of LDV. LDV replication may impair MuLV antigen synthesis as well as cellular protein synthesis, which results in c.p.e. An alternative reason may be the ratio of LDV antigens to MuLV antigens in infected cells. An excess of LDV antigens may interfere with the access of antibody to the MuLV antigenic determinants by steric hindrance. Also we do not know...
the reason for cells being MuLV-positive but LDV-negative in double staining. Even when LDV was inoculated onto MuLV-infected cells at a high m.o.i. (i.e. 1000 ID50/cell), only 50% at most of these became infected. Many factors could cause this low efficiency of infection, which awaits further study.

There were no significant differences between neurovirulent LDV-C and avirulent LDV-N, -Nu, -R and -P strains in their ability to replicate in macrophages both in vivo and in vitro (Table 4; Stroop & Brinton, 1983). It was shown here, however, that there was a large difference between these strains in their infectivities to MuLV-infected cells. LDV-C infected and replicated 50 to 100 times more than the avirulent strains in these cells. This might correspond to the capacity of LDV-C to replicate in the spinal cord of neuroparalysis-susceptible C58 mice and reflect the difference in their neurovirulence (Martinez et al., 1980). LDV-C adsorbed to MuLV-infected cells three times more efficiently than the avirulent strain, LDV-N. The higher infectivity of LDV-C to MuLV-infected cells may therefore be due to its greater ability to adsorb to the cells. LDV-N does not seem to be adsorbed specifically to MuLV-infected cells, since there was no difference in adsorption between MuLV-infected and uninfected cells. As the avirulent strains infected and replicated only in a small number of MuLV-infected cells (Table 4), it is likely that no significant specific binding of LDV-N could be detected in the adsorption test. It seems likely that the LDV receptor on MuLV-infected cells is different from that on macrophages. Ia antigens on macrophages have been reported to be receptors for LDV (Inada & Mims, 1984), but they may not be the major receptors (Buxton et al., 1988).

Endogenous ecotropic murine retroviruses are divided into two categories: N-tropic and B-tropic. The two types replicate well in NIH/3T3 and BALB/3T3 cells, respectively, but not vice versa (Pincus et al., 1971). The element which regulates this restriction is the Fe-I gene of the host cells. It was demonstrated that LDV could replicate in cells infected with MuLV irrespective of the MuLV tropism (Table 3). The incidence of neuroparalysis induced by LDV corresponds with the age of C58 mice; younger mice are more resistant to the disease (Duffey et al., 1976; Martinez et al., 1980). In leukemic-prone mice, N-tropic endogenous virus genes are expressed early in life and infectious viruses are detected in various tissues. The titre increases with age, but varies from organ to organ (Rowe & Pincus, 1972). Pease et al. (1982) have shown that in mice congenic at the Fe-I locus (introduction of the Fe-Ib gene into AKR mice), the rate of paralysis significantly decreased. This suggests the growth of endogenous retrovirus is essential for the induction of paralytic disease.

Chronic leukaemia develops in C58 mice as young as 6 months. This is postulated to be due to a leukaemogenic mink cell focus-forming (MCF) virus arising from recombination between N-tropic and xenotropic viruses (Staal et al., 1977). As paralysis is inducible by immunosuppressive treatment in mice younger than 6 months which have not yet become leukaemic, it is apparent that the leukaemia itself or MCF virus do not play an aetiological role.

It is important to know whether there is a correlation between MuLV gene expression and susceptibility to LDV infection in the spinal cord of C58 mice. Contag & Plagemann (1989) showed by the in situ hybridization technique that there are high levels of MuLV expression in the spinal cord from cyclophosphamide-treated C58 mice.

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References


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