Replication of Lactate Dehydrogenase-elevating Virus in C58 Mice and Quantification of Antiviral Antibodies and of Tissue Virus Levels as a Function of Development of Paralytic Disease

By WILLIAM A. CAFRUNY,*† CHARLES R. STRANCKE, KATHY KOWALCHYK AND PETER G. W. PLAGEMANN

Department of Microbiology, University of Minnesota School of Medicine, Minneapolis, Minnesota 55455, U.S.A.

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SUMMARY

Infection with the lactate dehydrogenase-elevating virus (LDV) triggers a generally fatal paralytic disease in old immunosuppressed C58 mice, but not in comparable mice of many other strains. We have compared the replication of LDV and the humoral immune response to it in C58 mice and mice of various resistant strains. Plasma LDV titres of persistently infected C58 mice were about tenfold higher than in other strains of mice and the proportion of LDV-permissive macrophages in peritoneal exudates of C58 mice was about twice as high as that observed in other mouse strains. C58 mice developed normal levels of anti-LDV IgG, as measured by ELISA, and normal levels of IgG that sensitized LDV to neutralization by rabbit anti-mouse IgG. C58 mice also developed normal IgM and IgG responses to human γ-globulin and sheep erythrocytes. The antibody responses to LDV were similarly inhibited by cyclophosphamide in C58 and resistant strains of mice, which enhanced the incidence of signs of paralysis only in C58 mice. Thus, the sensitivity of C58 mice to LDV-induced paralytic disease is not due to an inherent inability of the mice to mount a humoral antibody response to LDV, and a suppression of the antibody response by cyclophosphamide is not the only prerequisite for development of the disease. We have quantified LDV in various tissues of immunosuppressed and non-immunosuppressed, 8- or 9-month-old C58 mice as a function of time after LDV infection and in relation to the development of paralytic disease. Changes in tissue LDV titres as a function of time after infection paralleled those found in the plasma; LDV titres were highest 1 day post-infection, and then decreased to a lower persistent level during the next 1 to 2 weeks. Tissue LDV titres, including those of the spinal cord, were lower than those in the plasma, and our results indicate that most of the LDV in tissue homogenates may be attributable to blood contamination, even though the animals were extensively perfused before removal of the tissues.

INTRODUCTION

The lactate dehydrogenase-elevating virus (LDV) of mice replicates in a subpopulation of macrophages with appropriate cell surface receptors for LDV (Kowalchyk & Plagemann, 1985; Inada & Mims, 1984). This LDV-permissive subpopulation represents between 3 and 10% of the total resident peritoneal macrophages of Swiss and BALB/c mice (Tong et al., 1977; Stueckemann et al., 1982a). Upon primary infection of a mouse, the permissive cells in the host become productively infected, resulting in extensive viraemia, transient production of interferon, activation of the residual macrophages and the elevation of plasma lactate dehydrogenase (LDH) and a number of other plasma enzymes. The acute infection invariably...
progresses into a life-long, persistent infection associated with the presence of infectious LDV-antibody complexes (Notkins et al., 1966; Cafruny & Plagemann, 1982a; McDonald et al., 1983), continued elevation of plasma enzyme LDH levels (see Rowson & Mahy, 1975) and polyclonal activation of B cells (Cafruny & Plagemann, 1982a; Coutelier & Snick, 1985). The persistent infection is maintained by passage of LDV to newly formed permissive macrophages as they arise (Stueckemann et al., 1982b).

LDV is also the aetiologic agent of age-dependent polioencephalomyelitis (ADPE) in the C58 strain of mice (Murphy et al., 1980, 1983; Nawrocki et al., 1980; Martinez et al., 1980). Sensitivity to ADPE increases with age and is enhanced by immunosuppression treatment. This increased susceptibility seems to be related to a loss of T cell function (Murphy et al., 1983; Bentley & Morris, 1982; Bentley et al., 1983). Paralytic signs in these mice develop 2 to 3 weeks after infection with LDV. Histologically, ADPE is associated with infiltration by inflammatory cells and neuron destruction in the grey matter of the spinal cord and brain stem. In younger immunosuppressed C58 mice, LDV infection results in a histological polioencephalomyelitis without paralytic signs (Stroop & Brinton, 1983; Stroop et al., 1985).

A major question has been the role of the immune response in the pathogenesis of ADPE, and it is not yet clear whether there is an immunopathological component in the disease or whether susceptibility is due to lack of an effective antiviral immune response (Murphy et al., 1980, 1983; Nawrocki et al., 1980). In the present study, we have examined immune responses and LDV replication in the susceptible C58 strain and resistant strains of mice, and the results have been correlated with cyclophosphamide treatment and development of ADPE in susceptible mice. Some of our results have been reported in preliminary form (Cafruny & Plagemann, 1983).

METHODS

Mice. Outbred Swiss mice were obtained from BioLabs (St Paul, Mn., U.S.A.). BALB/c mice were either bred in the Department of Microbiology Animal Facility (BALB/cMn) or obtained from Jackson Laboratories (Bar Harbor, Me., U.S.A.; BALB/cJ). C58/M mice were obtained from Dr W. H. Murphy (University of Michigan, Ann Arbor, Mich., U.S.A.). C58/J, A/J, C3H/OUJ, CBA/J, C57BL/6J, DBA/2J and NZB/BLNJ mice were obtained from Jackson Laboratories. All mice used were female.

LDV. The neuroparalytic strain (LDV

Mouse perfusion and tissue preparations. Mice were injected intraperitoneally with 0·5 ml of a heparin solution (1000 units/ml), anaesthetized about 1 h later by injection with Nembutal and then perfused with about 75 ml phosphate-buffered saline (PBS, pH 7.4) containing 1 unit heparin/ml as described by Kascsak et al. (1983). A 25-gauge needle attached to an ISCO WIZ peristaltic pump was inserted into the right ventricle and a small incision was made in the left atrium. Perfusion was at a flow rate of about 4·5 ml/min, but not higher than 5 ml/min to avoid haemorrhage in the lung, and its efficiency was judged by blanching of the organs. The tissues were immediately removed, weighed, and rinsed and homogenized in PBS.

Antibody quantification and immunization. LDV-reactive antibodies present in the plasma of infected mice were quantified by ELISA as described previously (Cafruny & Plagemann, 1982a), using Linbro EIA microtitre plates (Flow Laboratories). The plates were coated for 1 h with 2 × 10⁸ to 1 × 10⁹ ID₅₀ of gradient-purified LDV per well in carbonate buffer pH 9·6. The plates were reacted with mouse plasma diluted in PBS-Tween 20 for 16 h at 4°C, followed by a 2 h incubation with alkaline phosphatase-conjugated goat anti-mouse IgG (Cappel Laboratories or Sigma) at dilutions of between 1:200 and 1:600. Wells were developed for 30 to 60 min with phosphatase substrate (Sigma no. 104) and read at 400 nm or 405 nm with a Beckman spectrophotometer or a Dynatech Minireader II. Plasma samples were generally assayed at dilutions of 1:50 to 1:300 or in duplicate at a dilution of either 1:100 or 1:200, which fell on the linear portion of standard titration curves (see Fig. 2).

Mice were injected intraperitoneally with 2·5 × 10⁷ sheep erythrocytes or 4 µg human γ-globulin at 0 time and 10 days later. IgM and IgG to both sheep erythrocytes and human γ-globulin in plasma were quantified by ELISA as described already, except that the plates were coated with a sonicated lysate of sheep erythrocytes (about 10 µg protein/well) or a solution of human γ-globulin (Cohn fraction II, Sigma; about 10 µg/well). For measuring IgM
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responses, a 1:300 dilution of alkaline phosphatase-conjugated goat anti-mouse IgM (Sigma) was substituted for the goat anti-mouse IgG.

**LDV-neutralizing and -sensitizing activity.** For the quantification of LDV complexes neutralizable by antibody to the IgG component of the complexes (sensitized immune complexes; Notkins et al., 1968), 100 µl samples of plasma from infected mice diluted 1:100 in PBS were incubated with either 20 µl normal rabbit plasma or 20 µl rabbit anti-mouse IgG (Sigma) at 37 °C for 30 to 60 min (Notkins et al., 1968). The samples were then assayed for infectious LDV as described above.

For the routine assay of neutralizing antibodies, plasma from LDV-infected or uninfected mice was shaken with an equal volume of ethyl ether for 3 min at room temperature (Notkins et al., 1968). The mixture was centrifuged, the ether layer was removed, the sample was extracted once more with ether and finally residual ether was evaporated at room temperature. Samples of ether-extracted plasma were mixed with equal volumes of a suspension of homologous LDV (about 10^{5.0} ID_{50}/ml). The mixtures were incubated at 37 °C for 1 to 2 h and then assayed for infectious LDV. The degree of neutralization was defined as the difference in LDV titre between samples incubated with ether-extracted immune and normal plasma. For the simultaneous assay of neutralizing and sensitizing antibodies, samples of ether-extracted plasma were incubated with an equal volume of a suspension of homologous LDV (10^8 to 10^9 ID_{50}/ml) at 37 °C for 1 to 2 h. The mixtures were then diluted 1:100 in PBS and samples thereof were incubated with normal rabbit plasma or rabbit anti-mouse IgG and then titrated for LDV as described above.

**Quantification of total plasma IgG.** The concentration of total IgG in plasma was determined by radial immunodiffusion using a kit purchased from Miles Laboratories (Cafruny & Plagemann, 1982a).

**RESULTS**

**Humoral immune response of C58 mice and mice from other strains to LDV**

Groups (three to five) of 2- to 3-month-old-Swiss, BALB/cMn, C57BL/6J, A/J, C3H/HeJ, CBA/J, DBA/2J, NZB and C58/J mice were infected with 10^6 ID_{50} of LDV_{MUR} and bled 19 and 215 days later. The pooled plasma samples were analysed for anti-LDV IgG, neutralizing antibodies, infectious LDV and total IgG. Mice of all strains responded with similar anti-LDV IgG responses with levels increasing between 19 and 215 days after infection. (A_{400} range 0.18 to 0.3 above control background measured at 1:200 plasma dilution.) LDV-neutralizing activity was not detectable in plasma of 19-day infected mice, but plasma from 215-day infected mice of all strains neutralized 10^{1.0} or 10^{1.5} ID_{50} of LDV_{MUR} during 1 h of incubation at 37 °C. The total plasma IgG levels of mice of all strains became elevated two- to fivefold after infection with LDV, consistent with the LDV-triggered polyclonal activation of B cells previously observed in BALB/c and Swiss mice (Cafruny & Plagemann, 1982a).

In Fig. 1, IgM and IgG responses to primary and secondary injections of sheep erythrocytes [2.5 × 10^9 erythrocytes intraperitoneally (i.p.)] and human gamma globulin (4 µg i.p.) are shown for Swiss, BALB/c, C58/M and AKR mice. Although slight differences in the magnitude of the responses were noted, these were statistically insignificant. The similarities in the patterns of response suggest that C58 mice respond normally to these heterologous antigens. In another experiment we immunized five 7-month-old C58/M mice with human γ-globulin. Both the IgM and IgG antibody responses were comparable to those in young C58 mice (data not shown).

**Humoral immune response of old C58 mice in relation to development of ADPE**

Groups of six young (2 to 4 months) or old (10 months) Swiss or C58/M mice were injected with cyclophosphamide and 1 day later these mice and groups of six untreated mice were infected with 10^6 ID_{50} LDV_{MUR}. The mice were bled 17 days post-infection and their pooled plasma was analysed for anti-LDV IgG and infectious LDV (Table 1). Five of six immunosuppressed and three of six non-immunosuppressed old C58 mice developed leg paralysis; all other mice appeared normal. The IgG anti-LDV antibody levels were higher in each group of C58 mice than in the corresponding group of Swiss mice. Although cyclophosphamide pretreatment suppressed the IgG anti-LDV response in both mouse strains, the cyclophosphamide-treated C58 mice of both age groups had antibody levels as high or higher than those found in non-immunosuppressed Swiss mice. Plasma LDV titres were, on average, about tenfold higher in C58 mice than in Swiss mice and higher in cyclophosphamide-treated than in untreated C58 mice.
In another experiment, we treated a group of ten 8-month-old C58/M mice with cyclophosphamide and 1 day later LDV-infected this group and a group of five companion mice not treated with the drug. At 3-day intervals, two mice of each group were bled (always the same mice) and their pooled plasma was titrated for anti-LDV IgG (Fig. 2) and infectious LDV (see below). The mice not treated with cyclophosphamide developed anti-LDV IgG within 3 days (Fig. 2a). The levels produced in immunosuppressed mice were lower and there was a 9-day lag before any anti-LDV IgG became detectable (Fig. 2b). The anti-LDV titres in cyclophosphamide-treated mice fluctuated between 12 and 24 days post-infection. Similar fluctuations have been observed between 6 and 15 days in BALB/c and Swiss mice not treated with cyclophosphamide by us (unpublished results) as well as by McDonald et al. (1983) and attributed to a switch from IgG1 to IgG2 production. Of the group of 10 cyclophosphamide-treated mice, eight developed paralytic disease between 16 and 28 days, whereas only one in five of the untreated mice did so. All of these mice were bled either at the height of developing paralytic signs or between 28 and 34 days post-infection, if they remained unaffected.

Neutralizing and sensitizing antibody responses

We have analysed plasma samples from these LDV-infected C58 mice for the capacity to neutralize exogenous standard virus, to sensitize exogenous virus to subsequent neutralization by anti-mouse IgG, and also with respect to the presence of endogenous sensitized virus (Table 2). Ether-extracted plasma from mice infected for up to 34 days failed to neutralize the infectivity of standard exogenous virus significantly at a concentration of 17% during 1 h incubation at 37°C. These results are consistent with a previous study employing ether-extracted plasma from LDV-infected CAF-1 mice (Notkins et al., 1966). Plasma from the C58 mice that was not extracted with ether similarly failed to neutralize the infectivity of exogenous LDV (data not shown).

Under the same conditions of initial incubation, however, ether-extracted plasma from LDV-infected C58 mice did sensitize standard virus to subsequent neutralization by anti-mouse IgG. This effect was demonstrable in plasma from C58 mice by 15 days after infection, regardless of
whether the mice were cyclophosphamide-treated or had developed ADPE, indicating the presence of LDV-reactive IgG in the plasma of all mice tested. Furthermore, sensitized virus–antibody complexes were observed in the plasma of untreated mice at 6 days, and in that of cyclophosphamide-treated ADPE-positive C58 mice at 24 days post-infection, as determined by the direct neutralization of their plasma LDV infectivity by incubation with anti-mouse IgG. The extent of neutralization of endogenous LDV infectivity by anti-mouse IgG was comparable to that observed with plasma of BALB/c mice 3 to 4 weeks post-infection (data not shown).

Comparison of LDV distribution in tissues of C58 mice with and without ADPE

Fig. 3 illustrates the plasma LDV titres of cyclophosphamide-treated and untreated C58/M mice, as well as of Swiss mice included as non-susceptible controls, as a function of time after infection with LDV. The plasma LDV titres of C58/M mice were slightly higher 1 day post-infection than those of Swiss mice and significantly higher than in Swiss mice during the persistent phase of infection.

We also determined LDV titres in the plasma, spinal cord, brain, liver and spleen of the nine C58 mice which were sacrificed and perfused at the height of paralytic disease and the six which were asymptomatic (Table 3). The LDV titres of plasma and tissues of the paralysed mice were 10- to 100-fold higher than those of the mice without symptoms, but it should be noted that the majority of the paralytic mice were cyclophosphamide-treated, whereas the opposite was the case for the asymptomatic mice. Thus, the higher tissue LDV titres may have correlated with
Table 1. Plasma anti-LDV IgG and infectious LDV and incidence of paralysis in old and young C58 and Swiss mice 17 days after infection with LDV<sub>MUR</sub>.

<table>
<thead>
<tr>
<th>Mice</th>
<th>Age (months)</th>
<th>Cyclophosphamide-treated</th>
<th>Anti-LDV IgG (A&lt;sub&gt;450&lt;/sub&gt;)</th>
<th>Plasma LDV (ID&lt;sub&gt;50&lt;/sub&gt;/ml)</th>
<th>Paralysed/total†</th>
</tr>
</thead>
<tbody>
<tr>
<td>C58</td>
<td>2-4</td>
<td>-</td>
<td>0.423</td>
<td>10&lt;sup&gt;6.5&lt;/sup&gt;</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>0.270</td>
<td>10&lt;sup&gt;7.5&lt;/sup&gt;</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>10-12</td>
<td>-</td>
<td>0.525</td>
<td>10&lt;sup&gt;7.0&lt;/sup&gt;</td>
<td>3/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>0.100</td>
<td>10&lt;sup&gt;8.0&lt;/sup&gt;</td>
<td>5/6</td>
</tr>
<tr>
<td>Swiss</td>
<td>2-4</td>
<td>-</td>
<td>0.093</td>
<td>10&lt;sup&gt;6.3&lt;/sup&gt;</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>0.021</td>
<td>10&lt;sup&gt;7.0&lt;/sup&gt;</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>10-12</td>
<td>-</td>
<td>0.277</td>
<td>10&lt;sup&gt;6.5&lt;/sup&gt;</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>0.039</td>
<td>10&lt;sup&gt;6.5&lt;/sup&gt;</td>
<td>0/6</td>
</tr>
</tbody>
</table>

* Groups of 12 young (2 to 3 months) or old (about 10 to 12 months) Swiss and C58/M mice were infected with 10<sup>6</sup> ID<sub>50</sub> of LDV<sub>MUR</sub> per mouse. Six of each group were injected with 200 mg cyclophosphamide/kg body weight 24 h before LDV infection. At 17 days after infection, the mice were bled, and the plasma was pooled for each group. Each pool was assayed for infectious LDV and a 1:200 dilution thereof by ELISA for anti-LDV IgG and the average absorbance from duplicate wells are shown. Values are corrected for absorbance observed with plasma from companion uninfected mice.

† Incidence of paralysis developing between 18 and 28 days post-infection.

Table 2. Neutralizing and sensitizing activity of plasma from LDV-infected C58 mice

<table>
<thead>
<tr>
<th>Plasma obtained</th>
<th>Change in LDV titre (log&lt;sub&gt;10&lt;/sub&gt; ID&lt;sub&gt;50&lt;/sub&gt;/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time post-infection (days) Neutralization Sensitization</td>
</tr>
<tr>
<td>Exogenous LDV*</td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>Control</td>
</tr>
<tr>
<td>LDV-infected + CY</td>
<td>+0.5</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>LDV-infected - CY</td>
<td>0</td>
</tr>
<tr>
<td>ADPE+, CY+</td>
<td>-0.5</td>
</tr>
<tr>
<td>ADPE-, CY−</td>
<td>-0.5</td>
</tr>
<tr>
<td>Endogenous LDV†</td>
<td></td>
</tr>
<tr>
<td>CY+</td>
<td>0</td>
</tr>
<tr>
<td>CY−</td>
<td>-1.5</td>
</tr>
<tr>
<td>CY−</td>
<td>-1.5</td>
</tr>
<tr>
<td>CY-24</td>
<td>-2.5</td>
</tr>
</tbody>
</table>

* Twenty μl of a suspension of LDV<sub>MUR</sub> (10<sup>7</sup> ID<sub>50</sub>/ml) was mixed with 100 μl PBS and 20 μl ether-extracted plasma from uninfected or LDV-infected mice (see Fig. 2). ADPE+, CY+ indicates a pool of plasma from six cyclophosphamide-treated mice bled at the height of developing paralytic symptoms, see text; ADPE−, CY− indicates a pool of plasma from three companion mice that were not treated with cyclophosphamide and did not develop symptoms. After 1 h incubation at 37 °C, the mixtures were diluted with 1:9 ml PBS. Then two 100 μl aliquots of each mixture were incubated with 20 μl of either normal rabbit plasma (neutralization) or of rabbit anti-mouse IgG (sensitization) at 37 °C for 35 min and then titrated for infectious LDV in mice. All values are expressed as differences from LDV incubated with plasma from uninfected mice (control). Differences in ID<sub>50</sub> of 0.5 log<sub>10</sub> or less were not considered significant.

† Plasma from LDV-infected C58 mice (see Fig. 2) was diluted 1:100 in PBS and 100 μl aliquots thereof were incubated with 20 μl of normal rabbit plasma or 20 μl of rabbit anti-mouse IgG at 37 °C for 1 h. Then the mixtures were titrated for infectious LDV in mice; the differences in virus titres are shown.

cyclophosphamide treatment rather than the development of ADPE. Furthermore, in both groups of mice, the tissue LDV titres were lower than those of the plasma.

In another experiment, individual 9-month-old C58/M mice were sacrificed and perfused at various times after infection, and their tissues titrated for LDV (Fig. 4). In general, the tissue LDV titres reflected those of the plasma but were lower than those of the plasma (compare Fig. 3 and 4). In mice not treated with cyclophosphamide, LDV levels in the central nervous system
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Fig. 3. Infectious LDV in plasma of 8-month-old, cyclophosphamide-treated (●) and untreated (○, △) C58 (○, ●) and 18-month-old Swiss (△) mice as a function of time after infection with LDV<sub>M</sub>. The details of the experiment are as in Fig. 2; four Swiss mice were infected and analysed in the same way as described there for C58 mice. Plasma samples were assayed for infectious LDV as described in Methods.

Table 3. LDV titres in tissues of infected C58/M mice with (+) and without (−) ADPE*

<table>
<thead>
<tr>
<th>ADPE</th>
<th>Cyclophosphamide-treated/total</th>
<th>Plasma</th>
<th>Liver</th>
<th>Spleen</th>
<th>Brain</th>
<th>Spinal cord</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>8/9</td>
<td>8.38 ± 0.12</td>
<td>7.06 ± 0.14</td>
<td>7.59 ± 0.15</td>
<td>6.23 ± 0.15</td>
<td>7.48 ± 0.13</td>
</tr>
<tr>
<td>−</td>
<td>2/6</td>
<td>6.73 ± 0.28</td>
<td>6.22 ± 0.30</td>
<td>6.58 ± 0.38</td>
<td>5.15 ± 0.10</td>
<td>5.55 ± 0.28</td>
</tr>
</tbody>
</table>

* The details of the experiment are described in the legend to Fig. 2 and the text. The mice were bled at the height of paralytic symptoms (between 16 and 28 days post-infection) or between 28 and 34 days if they remained asymptomatic. Then they were perfused, their tissues homogenized and the homogenates and the plasma were assayed for infectious LDV.

...tissues were highest at 1 day post-infection and then decreased to a lower level. Results comparable to those in Fig. 4(a) were obtained with BALB/c and Swiss mice (data not shown). The tissue LDV titres retained in cyclophosphamide-treated C58 mice were higher than those of untreated mice (Fig. 4), just as was observed for plasma LDV titres.

The results suggested that even after extensive perfusion of the mice, the LDV detected in tissues might be largely attributable to contamination with blood-borne virus. In order to test this hypothesis, mice were injected intravenously with a mixture of <sup>3</sup>H<sub>2</sub>O and [14C]inulin. At various times thereafter, individual mice were bled and their plasma was analysed for <sup>3</sup>H and <sup>14</sup>C. Some of the mice were perfused and their tissues then analysed for <sup>3</sup>H and <sup>14</sup>C, and the tissues of other mice were analysed for radioactivity without prior perfusion. <sup>3</sup>H<sub>2</sub>O is expected...
Fig. 4. LDV titres in various tissues of 9-month-old (b) cyclophosphamide-treated and (a) untreated C58/M mice as a function of time after infection with LDV. Six cyclophosphamide-treated and untreated C58/M mice were infected with 10⁶ ID₅₀ of LDV₄₅/6/mouse. At the indicated times, one mouse of each set was injected with heparin, anaesthetized 1 h later and perfused. Then its tissues were removed, weighed and homogenized in PBS, and the homogenates were assayed for infectious LDV. (a) ●, Brain; ▲, cervical cord; ▼, lumbar cord. (b) ●, Brain; ▲, spinal cord; ■, liver.

Table 4. Distribution of ³H and ¹⁴C in tissues of 9-month-old C58 mice after injection of ³H₂O and [¹⁴C]inulin

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Radioactivity/mg of tissue (% of plasma value)</th>
<th>Not perfused</th>
<th>Perfused†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>³H</td>
<td>¹⁴C</td>
<td>³H</td>
</tr>
<tr>
<td>Liver (3)</td>
<td>72</td>
<td>6.7</td>
<td>16</td>
</tr>
<tr>
<td>Spleen (2)</td>
<td>84</td>
<td>9.8</td>
<td>51</td>
</tr>
<tr>
<td>Brain (3)</td>
<td>86</td>
<td>1.7</td>
<td>39</td>
</tr>
<tr>
<td>Spinal cord (2)</td>
<td>87</td>
<td>4.5</td>
<td>18</td>
</tr>
</tbody>
</table>

* The mice were injected i.p. with 500 units heparin and about 1 h later intravenously with 0.5 ml of a solution containing 50 μCi ³H₂O and 10 μCi [¹⁴C]inulin/ml. The mice were bled and about 10 min after injection of the radiolabelled substances were, where indicated, anaesthetized and perfused as described in Methods. The tissues of the mice were removed and cut into the number of smaller segments indicated in parentheses. The pieces were each weighed and they, as well as samples of the plasma, were analysed for radioactivity. The averages of these values are presented. The plasma of individual mice contained between 200 and 300 d.p.m. of ³H and ¹⁴C per mg.
† The values for 1 and 2 are for two different perfused C58 mice.

To diffuse into tissues and equilibrate rapidly with intracellular and extracellular water spaces, whereas [¹⁴C]inulin is retained in the circulation (Oldendorf, 1971; Dawson & Segal, 1976) and thus can be used to estimate the amount of blood retained in tissues. We found that the distribution of ³H and ¹⁴C in tissues became about constant within 10 min after injection (data not shown). The level of ³H per g of tissue generally reached 60 to 90% of that of plasma, whereas the ¹⁴C concentration in tissues did not exceed 10% of that of plasma, but most often was much lower (see Table 4 for typical results), except in the kidney where it was 100 to 150% of that of plasma (data not shown). Perfusion of about 75 ml PBS containing heparin reduced the level of both ³H and ¹⁴C in the tissues to variable extent, but generally not more than 50 to 70% (Table 4), even though blanching of liver, heart and brain indicated successful perfusion. Both
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There appears to be a paucity of information regarding the immune responsiveness of C58 mice, even though this strain is an important model for the study of leukaemia as well as viral pathogenesis. Previous studies have shown that ageing C58 mice lose lymphocyte responsiveness to T cell mitogens (Lawton & Murphy, 1974; Schlabach et al., 1979), IgG memory may be impaired in these mice (Schott et al., 1981), and macrophage activation by some adjuvants is deficient in ageing C58 mice (Petrequin & Johnson, 1984). On the other hand, we found that the humoral antibody responses of C58 mice to unrelated conventional antigens and LDV infection were comparable to those of other mouse strains and at least some of the mice possessed normal levels of IgG anti-LDV within a few days of their death due to ADPE. C58 mice developing ADPE did not possess neutralizing antibodies, but the same was true for those C58 mice that did not develop paralytic signs during the first several weeks of infection whether or not they were cyclophosphamide-treated, or young or old, and this finding extends to mice of other strains (Notkins et al., 1966; Rowson et al., 1966). In any case, neutralizing antibodies which do develop in mice, including C58 mice, several months after infection have very low neutralizing efficiency when compared to rabbit anti-LDV antibodies (Cafruny & Plagemann, 1982a) and what role they may play in LDV infection and persistence is unclear. C58 mice did produce early IgG that sensitized endogenous as well as exogenously added LDV to neutralization by antimouse IgG. In some experiments the levels induced were slightly lower in cyclophosphamide-treated than in untreated mice. Cyclophosphamide treatment reduced the formation of IgG anti-LDV in these mice as measured by ELISA, but when individual mice were analysed, no correlation between levels of anti-LDV IgG and the development of paralytic disease was detectable. Furthermore, cyclophosphamide treatment had a similar inhibitory effect on anti-LDV IgG production in young C58 mice and mice of other strains without rendering them sensitive to ADPE. It is also of interest that ageing C58 and Swiss mice produced higher anti-LDV responses than younger mice of the corresponding strain. Therefore, it is unlikely that the sensitivity of C58 mice to LDV-triggered ADPE is solely due to a quantitative deficiency in the production of anti-LDV antibodies.

The role of immunosuppression by cyclophosphamide treatment in enhancing the sensitivity of C58 mice to LDV-triggered ADPE thus remains unresolved. It could be mediated by an effect on T suppressor cells, since cyclophosphamide is known to have selective toxicity for these cells, and in some cases fails to inhibit or may even enhance immune responses (Turk & Parker, 1982), and since the sensitivity of C58 mice to ADPE has been linked to the loss of a protective T lymphocyte subset with phenotypic characteristics of suppressor cells (Murphy et al., 1983; Bentley & Morris, 1982; Bentley et al., 1983). However, the protective function of these lymphocytes in ADPE has not been explained. Cyclophosphamide also enhances the sensitivity of BALB/c mice to experimental allergic encephalitis, an autoimmune disease which becomes apparent as hind leg paralysis 11 to 21 days after injection of myelin basic protein (Lando et al., 1980). These findings may suggest the involvement of an autoimmune pathogenesis for ADPE as originally proposed before an acute infection with LDV was implicated in triggering the disease (Duffey et al., 1976), but there is presently no experimental evidence which directly supports such a hypothesis.

Another potential mechanism of neuron damage in ADPE may be the replication of LDV in these cells (Brinton, 1981; Stroop & Brinton, 1983), which may be linked to the higher blood LDV titres in C58 mice relative to those in mice of other strains observed by us and other investigators (Brinton, 1981; Stroop & Brinton, 1983). Since these titres precede the appearance of neutralizing antibodies or anti-LDV antibodies detected by ELISA, they are independent of the anti-LDV antibody response, at least during the acute phase of infection. We have found that the proportion of LDV-permissive macrophages in peritoneal exudates of C58 mice is about twice that observed in Swiss mice (11.3 ± 3.2 compared to 5.6 ± 4.2% of the total; unpublished data). This may be the basis for a greater LDV production in C58 mice during the acute phase of
infection, but it is unclear how cyclophosphamide treatment results in the persistence of higher LDV levels in the blood and whether this influences the development of paralytic disease. Titrations of tissues from perfused C58 mice for infectious LDV did not yield any evidence for LDV replication in the central nervous system, specifically in the spinal cord. The higher LDV titres in the spinal cord of C58 mice developing paralytic symptoms correlated with cyclophosphamide treatment and higher plasma LDV levels as well as with the incidence of ADPE. We have not observed the specific appearance of LDV in the spinal cord of C58 mice developing paralytic symptoms which has been reported by Kascsak et al. (1983) and suggested a causal relationship between LDV replication in the spinal cord and ADPE. In agreement with the observations of Stroop & Brinton (1983) with young C58 mice, the LDV titres in various tissues, including the spinal cord, of old C58 mice were roughly proportional to those in plasma regardless of the time after infection. Also, they were generally lower than those of plasma whether or not the mice developed ADPE and our failure to remove \[^{14}C\]inulin efficiently from tissues by perfusion suggests that tissue LDV titres may mainly represent residual LDV in the circulation which was not removed by the perfusion. Only a 1 to 10% contamination of the tissues with blood-borne virus could account for most of the observed tissue LDV titres. It will be of interest to apply immunocytochemical and in situ hybridization techniques to search for LDV replication in the central nervous system and for the identification of any LDV-permissive cells.

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