Lack of Effect of Thymus and Spleen on the Incubation Period of Creutzfeldt-Jakob Disease in Mice

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(Accepted 27 January 1987)

SUMMARY

Genetically athymic and asplenic (Lasat), athymic (Nude), asplenic (Dh) or normal littermate (Hetero) mice with a BALB/c genetic background were injected either intracerebrally or intraperitoneally with a 1% or 10% homogenate of mouse brains infected with the Fukuoka 1 strain of the Creutzfeldt-Jakob disease (CJD) agent. As there were no significant differences in incubation periods among the five groups (Lasat, Nude, Dh, Hetero and BALB/c) inoculated with the same dilution, via the same route, it was concluded that cell-mediated immunity dependent on the thymus plays no significant role in host defence against the CJD agent, and the spleen, a critical site of agent replication, is apparently not an obligatory source from which infection spreads to the central nervous system.

Creutzfeldt-Jakob disease (CJD) is a slow, clinically progressive spongiform encephalopathy caused by an unconventional infectious agent similar to kuru, scrapie or transmissible mink encephalopathy (Gajdusek, 1977). Following successful transmission of the agent to small rodents (Manuelidis et al., 1978; Tateishi et al., 1979), CJD has been extensively studied. Nevertheless, little is known of the role of the immune system in pathogenesis. It has been well established that the causative agent in scrapie replicates in the lymphoreticular system, notably in the spleen (Fraser & Dickinson, 1970, 1978; Clarke & Haig, 1971; Collis & Kimberlin, 1985; Bruce, 1985) and that it produces lesions in the nervous system in the absence of any detectable host immune response (Kingsbury et al., 1981).

To clarify the role of the thymus and the spleen in an experimental model of CJD in mice, the present studies were carried out using genetically athymic and asplenic mice 'Lasat' (nu/nu, Dh/+ ) and their littermates 'Nude' (nu/nu, +/+), asplenic 'Dh' (nu/+ , Dh/+ ) and normal 'Hetero' (nu/+ , +/+), maintained in our laboratory by backcross matings with BALB/c nude mice. The inbred strain of BALB/c (+/+ , +/+ ) mice was purchased from Seiwa Experimental Animals Ltd, Yoshitomi, Japan. The breeding of these mice and the experiments were carried out under specific pathogen-free conditions using isolators or barrier systems.

The CJD strain Fukuoka 1, isolated in mice from the brain of a patient with CJD (Tateushi et al., 1979), was used. The inoculum was obtained from B10.D2 mice with advanced clinical CJD. The brains [10^7.4 LD50 mouse intracerebral (i.c.) units/g wet weight] were homogenized in phosphate-buffered saline (PBS), and centrifuged at 2000 g for 10 min. Twenty µl of the 1% (w/v) supernatant was inoculated by the i.c. route into each weanling mouse, and 50 µl of the 10% or 1% supernatant was inoculated by the intraperitoneal (i.p.) route. Mice were examined two or three times a week for clinical evaluation of CJD which began with ruffled fur, arched back, slow righting-reflex, tail plasticity, incoordinated movements and bradykinesia followed by ataxia and paraplegia in the hind legs. The disorders progressed rapidly, and the mice usually died within 4 weeks after onset. The mice were anaesthetized with ether and sacrificed by decapitation when they were in extremis or had shown severe clinical signs of CJD for 3 consecutive weeks. The incubation periods were defined as the interval between inoculation and sacrifice. The brains were fixed in 10% buffered formalin, embedded in paraffin, sectioned at

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Table 1. Incubation period of CJD in mice

<table>
<thead>
<tr>
<th>Route of infection</th>
<th>Dilution of brain homogenate (%</th>
<th>Hetero Nude</th>
<th>Asplenic Lasat</th>
<th>BALB/c Male</th>
<th>BALB/c Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.p. 1</td>
<td></td>
<td></td>
<td></td>
<td>(n=4)</td>
<td>(n=7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>279 ± 29*</td>
<td>277 ± 23</td>
<td>288 ± 13</td>
<td>268 ± 21</td>
</tr>
<tr>
<td>I.c. 1</td>
<td></td>
<td></td>
<td></td>
<td>(n=6)</td>
<td>(n=8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>262 ± 43</td>
<td>252 ± 24</td>
<td>245 ± 39</td>
<td>243 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=7)</td>
<td>(n=9)</td>
<td>(n=10)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>I.c. 10</td>
<td></td>
<td>148 ± 8</td>
<td>145 ± 12</td>
<td>143 ± 21</td>
<td>140 ± 3</td>
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<tr>
<td></td>
<td></td>
<td>(n=2)</td>
<td>(n=6)</td>
<td>(n=10)</td>
<td>(n=4)</td>
</tr>
</tbody>
</table>

* Values given are the mean value (days) ± S.D.; n is the number of affected animals.
† Ni, Not inoculated.

6 μm thickness and stained with haematoxylin and eosin for light microscopy. The clinical diagnosis of CJD was confirmed by histopathological examination of brain in which a severe spongy state and proliferation of astrocytes were observed especially in the white matter of the cerebrum, brain stem, cerebellum, internal capsule and optic and olfactory tracts, as reported previously (Tateishi et al., 1980). The clinical features and the pathological changes were essentially the same in all animals.

Table 1 shows the incubation period in Lasat mice, their littermates and BALB/c mice following i.p. or i.c. injection of CJD agent. The length of incubation for male and female BALB/c mice was identical; the incubation periods for male and female Lasat mice and their littermates are similar (not shown) which is contrary to the findings of Kingsbury et al. (1983) who demonstrated sex-related differences in the incubation period of CJD in B10.AKM, C3H/SwSn and C3H/DiSn mice. No significant differences were observed among the incubation periods in each mouse strain. Similarly, when animals were injected i.p. with the 1% or 10% dilution or i.c. with the 1% dilution, there were no significant differences (P > 0.05) in the mean incubation period in any of the strains (Table 1). This is the first evidence that i.c. or i.p. injection into athymic, asplenic and athymic-asplenic mice failed to influence the incubation periods in experimental CJD.

It has been reported that the T cell system plays no role in host defence mechanisms to scrapie or in the pathogenesis of experimental scrapie. This is based on the observations that thymectomy and irradiation (McFarlin et al., 1971) or neonatal thymectomy (Fraser & Dickinson, 1978) failed to influence the incubation period after peripheral scrapie injection. In experimental CJD, there is only one report demonstrating in athymic nude mice a similar incubation period to that of the normal mice after i.c. injection (Tateishi et al., 1980). It is evident from the present findings that neither the thymus nor the spleen has any effect on the incubation period. In addition, Lasat mice are particularly immunodeficient and have a lower γ-globulin level than their Nude or Dh littermates (Machado et al., 1976), but they developed CJD after the same incubation period. These results suggest that cell-mediated immunity plays no role in the pathogenesis of mouse CJD.

The spleen is an important site of replication of the unconventional infectious agents. Infectivity of scrapie rose to reach a peak in the spleen of mice before it appeared in the brain, following extraneural injection (Dickinson & Outram, 1979; Kimberlin & Walker, 1979; Collis & Kimberlin, 1985; Bruce, 1985); and high infectivity was also detected in the spleen of mice infected with CJD (Tateishi et al., 1980; Kuroda et al., 1983). Splenectomy before infection or genetic asplenia prolonged the incubation period of scrapie in mice after i.p. but not i.c. injection (Fraser & Dickinson, 1970, 1978; Clarke & Haig, 1971; Dickinson & Fraser, 1972), whereas splenectomy of hamsters had no effect on the incubation period after i.p. injection (Kimberlin & Walker, 1977, 1986). In contrast to scrapie, the present studies demonstrate that regardless of whether or not mice have the asplenic Dh gene, there are no differences in the incubation period of CJD, both in the case of i.p. and i.c. injection. This finding suggests that the spleen is not an obligatory source from which infection spreads to the central nervous
system. The lack of effect of splenectomy on the incubation period in scrapie may be due to the presence of sufficient non-splenic extraneural sites or lymphoid organs for both the uptake and multiplication of the agent. Kimberlin & Walker (1986) reported the possibility of direct infection of nerve tissue from the peritoneum and transport to the thoracic cord in hamsters following i.p. injection with high doses of scrapie. The present findings on CJD seem to support this proposal. However, other possible routes by which the agent invades the central nervous system after i.p. injection have to be excluded, for example, uptake and transport via migrating cells, cell-to-cell transfer involving both non-specific phagocytosis and pinocytosis, and specific receptor-binding mechanisms.

We thank Dr A. Takenaka for advice and encouragement, and K. Hatanaka for excellent technical assistance.

REFERENCES


(Received 30 October 1986)