Prolongation of Scrapie Incubation Period by an Injection of Dextran Sulphate 500 within the Month Before or After Infection

By C. F. FARQUHAR* and A. G. DICKINSON
AFRC & MRC Neuropathogenesis Unit, West Mains Road, Edinburgh EH9 3JF, U.K.

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SUMMARY

A single intraperitoneal injection of 250 μg dextran sulphate 500 (DS500) reduced the susceptibility of mice to scrapie given by the same route. A lower dose (25 μg) was less effective but still produced significant incubation period lengthening, while a high dose (2.5 mg) further increased the degree of prolongation. This reduced susceptibility occurred with DS500 administered up to at least 4 weeks prior to intraperitoneal scrapie inoculation and up to at least 2 weeks after scrapie inoculation. A reduced average effect, but more variable between mice, was obtained with DS500 given 1 month or 2 months after scrapie. The effective scrapie titre was reduced by 90% when DS500 was injected either 72 h before or 7 h after ME7 scrapie. Using a relatively lower but normally still fatal dose of the 22A strain of scrapie approximately 50% of the treated mice survived. The effective 90% loss of titre was consistent with either of these strains of scrapie in 11 different inbred strains of mice (BALB/c, BSC, BRVR, C3H, C57BL, IM, LM, MM, RIII, VL and VM). No significant increase in the prolongation effect was obtained using multiple DS500 doses in two different time combinations.

DS500 causes long-term interference in both the early processing and the replication of scrapie agent, unlike those immunomodulators which increase susceptibility.

INTRODUCTION

When an inoculum containing scrapie agent is injected intraperitoneally (i.p.) into inbred mice the infectivity titre rises in lymphoreticular organs, such as the spleen, prior to its increase in the central nervous system (CNS) (Eklund et al., 1967; Dickinson & Fraser, 1969). In surgically splenectomized mice (Fraser & Dickinson, 1970, 1978) the onset of clinical disease is delayed, probably as a result of a reduction in available replication sites for the agent (Outram, 1976).

Many pharmacological (Kimberlin & Walker, 1979b, 1983) and immunomodulatory (Gresser & Pattison, 1968; Worthington & Clark, 1971; Outram et al., 1974, 1975; Dickinson et al., 1978; Kimberlin & Cunnington, 1978; Ehlers et al., 1984; Ehlers & Diringer, 1984; R. H. Kimberlin & C. A. Walker, unpublished) compounds have been used to elucidate the role of the lymphoreticular system in the early pathogenesis of the peripherally routed infection. For example, the murine T lymphocyte mitogen, phytohaemagglutinin (PHA) consistently increases the operational titre of scrapie by between 0.5 and 1.5 log10 LD50 units when both the lectin and agent are administered i.p. (Dickinson et al., 1978). Host susceptibility is increased most when PHA injection occurs within the 3 h prior to scrapie inoculation. If given at the same time as scrapie the effect is reduced, and if given an hour or more after scrapie injection the effect on titre and on incubation period is negligible. The narrowness of the 'time window' when PHA and other immunoregulatory substances can affect pathogenesis indicates that they act on a primary or very early event in the establishment of infection. Two distinct types of mechanism may be responsible: (i) they either facilitate entry into the initial cell type involved in establishing infection, or restrict access to putative agent-degrading cells or (ii) they increase or decrease the available number of such cells without altering in any way the actual agent–cell interaction. Whatever the details of the action of PHA, its ability to alter scrapie pathogenesis...
ends very abruptly. If treatments are found which change the dynamics of scrapie incubation when used days after the injection of scrapie agent this would indicate that processes later than the initial infection were being altered, for example the subsequent transfer of infectivity from cell to cell.

METHODS

Mouse strains. The following inbred strains homozygous for Sinc\(^{s7}\) were used: BALB/c/LacDk, BRVR/SrDk, BSC/Dk, C3H/FaDk, C57BL/FaDk, LM/Dk, MM/Dk, R11Ho/FaDk and VL/Dk. Two Sinc\(^{p7}\) strains, VM/Dk and IM/Dk were also included. Sinc is the major gene contributing to the overall dynamics of scrapie replication in mice. The magnitude of its effect for some strains of scrapie is such that the difference in incubation period between the two homozygotes (s7s7 and p7p7) is in the order of a third or more of a murine lifespan. Sinc operates at an extraneural level, exerting control over replicative events in the lymphoreticular system, as well as at a neural one. All animals were over 6 weeks of age at scrapie injection and individual treatment groups were matched for age and sex. The use of many strains of mice was a deliberate attempt to establish the generality of the findings and to search for any genetic difference in the dextran sulphate 500 (DS500) response.

Scrapie inocula. Scrapie strain ME7 was cloned by passage from experiments involving serial 10-fold dilutions of terminal scrapie brain in C57BL mice and using a late incubation case in the endpoint dilution group. The same procedure was carried out for 22A strain scrapie but passage was through VM mice. Frozen brain (− 30 °C) was thawed and homogenized in pyrogen-free saline [NaCl, B.P. 0.9 °o (w/v), McCarthy]. This was centrifuged at 2000 \(g\) for 10 to 15 min and the supernatant diluted in pyrogen-free saline as necessary: 0.02 ml was injected i.p. with a 26-gauge needle. Injections were done close to the same time each day to minimize diurnal variation.

The titre estimates in serial dilution experiments were calculated using the Karber method; other titres were estimated from the incubation period by reference to strictly comparable standard dose-response curves in other experiments.

Treatment groups. DS500 (sodium salt, Sigma) was dissolved in pyrogen-free saline and filtered through a 0.22 \(\mu\)m Millex filter (Millipore). Unless stated otherwise, 0.02 ml of 12.5 mg/ml DS500 solution was injected i.p. Control groups were either uninjected or injected i.p. with the same volume of pyrogen-free saline.

Coulter counter analysis and histology. Peritoneal exudate cells (PEC) were obtained by peritoneal lavage with cold HEPES-buffered Hanks' balanced salt solution (HBSS, Gibco) pH 6.7, and maintained on ice in siliconized glass centrifuge tubes. The volume of HBSS used was adjusted according to the weight of the animal and 0.2 ml of the resulting cell suspension was added to 12.3 ml isoton (Coulter Electronics, Luton, U.K.) in a Coulter vial on ice. Profiles of cell size against cell numbers were obtained with a Coulter counter ZB1 and Channelyser. In order to minimize the known complication of diurnal variation, all PEC were collected by lavage at 2 p.m. At the same time slides were prepared using a cytocentrifuge (Cytospin, Shandon Southern Products, Runcorn, U.K.; 1000 r.p.m., 10 rain) with a range of dilutions of the PEC. The Cytospin preparations were stained with 'Diff-Quik' and the cell populations identified and counted.

Clinical scoring and pathology. Incubation periods for individual coded animals were obtained as previously described (Dickinson et al., 1968). The scrapie diagnosis was confirmed histologically.

RESULTS

Several studies were carried out without scrapie injection to investigate the dynamics of the cell populations induced within the peritoneum by either DS500 or PHA i.p. While it was appreciated that these cell populations might have little to do with the processing of infectivity it was considered important to have some appreciation of the changing cellular environment into which the inoculum was to be placed. A study of the routing of i.p. injected inoculum with radiolabelled liposomes indicated that 40% of the label was retained for at least 30 min in the peritoneum (Millson et al., 1979).

Peritoneal cell response to DS500 alone

C3H and BRVR inbred mice of both sexes and a range of ages were injected i.p. with 100 \(\mu\)g to 2.5 mg of DS500. These doses were chosen to be around the known mitogenic intravenous dose of 250 \(\mu\)g/mouse (Gronowicz & Coutinho, 1974). Doses of 0.5 mg/mouse and above were rejected for use in subsequent work because of an unacceptably high incidence of localized internal haemorrhaging and excessive thinning of the peritoneal wall around the site of injection.
Table 1. Effect of injecting DS500 i.p. before or after injecting ME7 scrapie i.p. into C3H and RIII inbred mice*

<table>
<thead>
<tr>
<th>Timing of DS500 injection before (-) or after (+) scrapie injection</th>
<th>C3H</th>
<th>RIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>-4 weeks before scrapie injection</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>-2 weeks before scrapie injection</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>-1 week before scrapie injection</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>-5 days before scrapie injection</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>-4 days before scrapie injection</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>-3 days before scrapie injection</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>-2 days before scrapie injection</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>-1 day before scrapie injection</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>-3 h before scrapie injection</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>-3 h (replicate)</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>+3 h after scrapie injection</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>+7 h after scrapie injection</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>+17 h after scrapie injection</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>+1 day after scrapie injection</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>+2 days after scrapie injection</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>+3 days after scrapie injection</td>
<td>ND</td>
<td>24</td>
</tr>
<tr>
<td>+4 days after scrapie injection</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>+2 weeks after scrapie injection</td>
<td>ND</td>
<td>10</td>
</tr>
</tbody>
</table>

* 0.02 ml of 1% brain homogenate, 500 g supernatant; 50 i.p. LD50 units of ME7.
† Significance of difference from pyrogen-free saline-injected controls which had an incubation period (days) of 315 ± 5 for the C3H mice and 303 ± 5 or 290 ± 4 for the RIII mice; combined results of two experiments. Three to 12 mice per group. t test. P = 0.05 (X) or <0.01 (XX); NS, not significant. ND, Not done.

However, the only deaths in all the experiments involved about 50% of animals given a single 2.5 mg dose of DS500 and in one animal given four weekly doses each of 0.5 mg DS500. For most experimental purposes a standard regime of 250 μg/mouse was used.

The cellular composition of peritoneal exudates varied little with dose but widely with interval after injection of DS500. PEC from DS500-injected and control, pyrogen-free saline-injected animals were indistinguishable until 5 and 7 h post-injection when the PEC from DS500-treated animals displayed increasing numbers of polymorphonuclear neutrophils (PMN) and small macrophages. PMN numbers peaked at 12 h with total cell numbers reaching a maximum 24 h after DS500 injection. By 48 h PMN and small macrophage numbers had returned to control values whereas large numbers of degenerating phagocytic cells were not cleared from the system until 22 h after DS500 treatment when PEC histologically and numerically resembled control populations.

Peritoneal cell response to PHA alone

Adult male C3H inbred mice were used. All treated animals followed similar response patterns over a time course tested at 1, 2, 3, 4 and 7 h and 1, 2, 5 and 14 days. Although 1 h after injection total PEC numbers decreased, this was followed by the rapid accumulation of PMN, their numbers peaking at 7 h. A slower rise in macrophage numbers resulted in a peak in total PEC at 24 h, although small lymphocyte numbers contained to increase, with a concomitant decrease in large macrophages, over the 4 to 5 days after PHA injection. Control numbers and cell population distributions were resumed by 14 days.

All the peritoneal responses to DS500 and PHA were unaffected by the age, sex or mouse strain used.

Effects of DS500 on scrapie incubation

The effect on scrapie incubation of giving DS500 i.p. over a range of times prior to injecting scrapie inocula i.p. was studied initially as, in our experience, immunomodulators had usually only been effective if administered within the few hours before scrapie. Some groups of animals were included where DS500 was given after scrapie injection. The results, shown in Table 1,
indicating that a significant prolongation of scrapie incubation period was achieved in most
groups where DS500 was given prior to scrapie. Also, the treatment was effective over a much
longer time course than obtained using PHA or lipopolysaccharide (LPS) to shorten the scrapie
incubation period. Most surprisingly, treatment was equally effective when DS500 was
administered 7 h after ME7 scrapie leading to the subsequent use of a longer range of time
intervals, and the discovery that this prolongation effect was demonstrable when DS500 was
given 2 weeks after ME7.

The results (Table 1) also show differences between mouse strains in the amplitude and timing
of the DS500-induced alteration in ME7 incubation period. There was a greater prolongation
effect in RIII mice than in C3H mice, the only exceptions being the groups injected 48 h prior to
and 3 h after ME7. The results also indicate a difference in the dynamics of the interactions
between the ME7 inoculum and the DS500-induced changes in these two strains of mice. For
example, with the RIII mice there were two high response peaks, with a trough when DS500 was
given in the 2 days before ME7.

To investigate whether these two mouse strains represented extremes of the interaction
between DS500 and the ME7 scrapie agent, other inbred mouse lines, which like C3H and RIII
mice were all homozygous for Sinc<sup>57</sup>, were tested. DS500 was given 7 h after scrapie because this
regime produced good prolongation effects in both C3H and RIII mice. In all strains tested
DS500 treatment lengthened scrapie incubation period, but there was appreciable variation in
this between experiments involving the same mouse strains (Table 2). This was not attributable
to any immaturity of the animals at the time of injection because a standard adult age range was
used and within this range there was no evidence that age had any effect on the variation.

Table 3 shows that decreasing susceptibility to ME7 scrapie depended on the dose of DS500.
In both RIII and C3H mice the smaller doses of DS500 reduced the prolongation effect while the
larger doses augmented it. Multiple DS500 injections did not significantly affect the
prolongation compared with a single injection. In C3H mice given four weekly doses of DS500
and injected with a 10% ME7 brain homogenate 24 h later, the mean incubation period was
336 ± 14 days compared with a control value of 313 ± 6 days for a single injection. The same
strain given DS500 7 and 19 h after scrapie produced a mean incubation period of 310 ± 7 days.
This was the only group in any of our experiments where DS500 proved ineffective in prolonging
incubation period.

In all of the above experiments every animal given scrapie became clinically affected, whether or not DS500 was administered. In another model, that of 22A scrapie in VM and IM
inbred mice (both Sinc<sup>57</sup>) there were survivors (Table 4). By reference to standard dose—response
curves for 22A in VM mice, the dose of 22A used was 10 i.p. LD<sub>50</sub> units and the partial
survivorship in the DS500 groups indicated a 90% reduction of effective titre. In none of the
ME7 experiments was the dose used less than 50 i.p. LD<sub>50</sub> units: the 90% titre reduction
produced by DS500 therefore still left a fully infective dose.

ME7 scrapie was titrated i.p. in C3H and VL mice given DS500 7 h after scrapie injection and
also in VL mice given DS500 3 days prior to scrapie. All the titrations showed a 90% reduction of
the infectivity titre (Fig. 1). With DS500 given to VL mice 72 h before infection, the reduction of
**Effect of DS500 on scrapie incubation period**

Table 3. *Effect of different doses of DS500 on incubation period of ME7 scrapie*

<table>
<thead>
<tr>
<th>DS500 (mg/mouse)</th>
<th>Incubation period (days ± S.E.)</th>
<th>DS500 7 h after 3000 i.p.</th>
<th>Incubation period (days ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrogen-free saline</td>
<td>243 ± 1</td>
<td>Pyrogen-free saline</td>
<td>299 ± 6</td>
</tr>
<tr>
<td>0.025</td>
<td>261 ± 10</td>
<td>0.2</td>
<td>346 ± 7</td>
</tr>
<tr>
<td>0.25</td>
<td>307 ± 15</td>
<td>0.25</td>
<td>349 ± 8</td>
</tr>
<tr>
<td>2.5</td>
<td>395 ± 5</td>
<td>0.3</td>
<td>350 ± 11</td>
</tr>
<tr>
<td>0.5</td>
<td>366 ± 9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Three to 12 mice per group.

Table 4. *Effect of a single DS500 injection i.p. on the incubation period of 22A scrapie i.p. in Sicp inbred mice*.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Incubation period (days ± S.E.)</th>
<th>Survivors (%)</th>
<th>Incubation period (days ± S.E.)</th>
<th>Survivors (%)</th>
<th>Incubation period prolongation (°)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VM</td>
<td>486 ± 8</td>
<td>58</td>
<td>378 ± 4</td>
<td>0</td>
<td>29</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IM</td>
<td>555 ± 24</td>
<td>44</td>
<td>458 ± 8</td>
<td>0</td>
<td>21</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Twelve to 14 mice per group. Animals were injected with DS500 or saline 7 h after scrapie: 10 i.p. LD50 units of 22A.

Fig. 1. Titration of ME7 i.p. in C3H and VL mice treated with DS500 or pyrogen-free saline i.p. at -72 h and +7 h (titre estimates given in text). C3H + 7 h, △ saline, ▲ DS500; VL + 7 h, ○ saline, ● DS500; VL - 72 h, □ saline, ■ DS500. Broken lines link to points with <50% scrapie incidence.

titre was 1·2 log10 i.p. LD50 units from the control value of 105·8/g of brain. With DS500 given 7 h after the scrapie injection the reduction was 0·7 log10 i.p. LD50 units in VL mice from a control value of 105·8, and in C3H mice the reduction was 0·8 log10 i.p. LD50 units from a control value of 105·8. It is rather unlikely that these three titre changes were significantly different because general experience with scrapie titration has shown that differences need to be in excess of 0·5 log10 units to be repeatable. All three DS500 titration curves in Fig. 1. are displaced to the right by 0·25 to 0·5 log10 units which suggests that the treatment slightly alters the relationship of scrapie dose and incubation period.
Table 5. Effect of combined DS500 and PHA treatment on i.p. ME7 scrapie* in C3H mice

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Incubation period (days ± S.E.)</th>
<th>Difference from control (%o)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrogen-free saline control</td>
<td>302 ± 3</td>
<td>-11</td>
<td>XX</td>
</tr>
<tr>
<td>-3 h PHA</td>
<td>269 ± 3</td>
<td>-11</td>
<td>XX</td>
</tr>
<tr>
<td>-72 h DS500</td>
<td>329 ± 9</td>
<td>+9</td>
<td>XX</td>
</tr>
<tr>
<td>+7 h DS500</td>
<td>342 ± 4</td>
<td>+13</td>
<td>XX</td>
</tr>
<tr>
<td>-72 h DS500 and -3 h PHA</td>
<td>310 ± 4</td>
<td>+3</td>
<td>NS</td>
</tr>
<tr>
<td>-3 h PHA and +7 h DS500</td>
<td>306 ± 5</td>
<td>+1</td>
<td>NS</td>
</tr>
</tbody>
</table>

* 100 i.p. LD50 units of ME7; eight to 13 mice per group.
† XX, <0.01; NS, not significant.

At the standard doses used here, DS500 and PHA had an approximately equal effect on incubation period but in opposite directions; therefore, their combined effect was examined. From the results they worked additively and it appeared to be of no consequence which substance was injected first (Table 5).

That DS500 affected scrapie incubation when administered a few hours after scrapie injection could be understood as an effect on the initial uptake and processing of the infectivity, although DS500 is effective at +7 h whereas PHA is not effective at +1 h (Dickinson et al., 1978). However, the effectiveness of DS500 injected 2 weeks after scrapie suggested modulation at later levels of agent–cell interaction than initial uptake and susceptibility. In two experiments the DS500 dose was given either at 1 month or 2 months after scrapie injection. At this time the replication in spleen is known to have reached the plateau level of infectivity (Dickinson & Fraser, 1969; Hunter et al., 1972). Fig. 2 shows that some individuals at these long time intervals may be unaffected by the DS500 but that at both time intervals more than half the animals had prolonged incubation periods, exceeding any of the individual values in the control group. They were at least as prolonged as the +7 h groups in other experiments, as shown in the figure.

Analysis of the scrapie lesion profiles from all the experiments reported here revealed no differences between groups treated with DS500, PHA or pyrogen-free saline.
Effect of DS500 on scrapie incubation period

DISCUSSION

A single i.p. dose of DS500 reduces susceptibility to scrapie infection. This results from an effective titre loss of up to 90% compared with pyrogen-free saline treated controls. Our previous experience of treatment with the conventional immunomodulators PHA and LPS has established the opposite effect, i.e. shortening of incubation period with a concomitant increase in effective titre (Dickinson et al., 1978).

As outlined in the Introduction a similar lengthening of incubation period has also been described in surgically splenectomized (Fraser & Dickinson, 1970, 1978) and genetically asplenic (Fraser & Dickinson, 1978) mice with peripherally routed scrapie. In these experiments, however, no loss of titre occurred, the increase in incubation period being due to a delay in early events in pathogenesis (Outram, 1976; A. G. Dickinson & H. Fraser, unpublished). The authors considered that sites important for the early replicative events had been depleted.

DS500 interferes with pathogenesis over a wide range of times around scrapie injection i.p. This is quite unlike the action of PHA, LPS and methanol extraction residue of BCG in shortening incubation (Dickinson et al., 1978; Kimberlin & Cunnington, 1978; C. F. Farquhar, unpublished) or of the antiviral compound HPA-23 (Kimberlin & Walker, 1979b, 1983) in prolonging it, all of which operate over a much narrower time range. Most surprisingly of all, a DS500 treatment given weeks after scrapie injection is still found to alter incubation period although we do not yet know whether this also is due to loss of titre. The long time range of the DS500 effect could well be due to its mode of action being different for early and late administration. Our results differ from those of Ehlers & Diringer (1984) who found a higher delay peak which rapidly declined when DS500 was given later than 3 days after scrapie infection. In contrast, during this period the response in our experiments remained more consistent even though it involved a DS500 dose level at which Ehlers & Diringer detected no effects: mouse strain differences probably contribute to this disparity, as may scrapie strain differences.

HPA-23, like DS500, is effective in prolonging the incubation period of peripherally routed scrapie when administered after scrapie injection. There does, however, appear to be a considerable difference in the underlying mechanisms involved. HPA-23 was administered most effectively by multiple injection either before or after scrapie infection, and while treatment after scrapie inoculation appeared more effective than those before, HPA-23 was ineffective when given more than 48 h after scrapie inoculation.

DS500 is, however, like PHA, LPS and HPA-23 in that it causes a significant change in infective titre, indeed producing a change of the same magnitude although in the opposite direction from the other two mitogens. When PHA and DS500 are administered at their respective optimal times in the same animal their individual effects are cancelled out, giving an incubation period similar to that in controls. DS500 either acts oppositely on some of the stages previously activated or enhanced by PHA, or operates on quite different cell lineages, or both. It is interesting to note too that even when DS500 is given before PHA the combined result is the same. Study of the numbers and histology of PEC after DS500 and PHA injection into the peritoneum gives no clue to the identity of the cell types important in altering pathogenesis. As PHA was injected 3 h prior to scrapie agent in the present experiments the inoculum always mixed with a rapidly increasing population of PMN while other cell types appeared stable. This situation existed whether or not PHA was given on its own or in combination with DS500 injected either 3 days prior to PHA or 7 h after scrapie.

Although DS500 is an effective modulator of scrapie pathogenesis, no simple picture has emerged of the level at which it operates, and there is more than a suggestion that different mechanisms are involved. The multiplicity of direct and indirect effects of DS500 in vivo must be considered especially in association with different treatment timings throughout the course of infection. Its acidic groups interact with circulating proteins of the complement, fibrinolytic, kinin and coagulation cascades (Walton, 1954; Loos & Bitter-Suermann, 1976) and by increasing the net negative charge on cell membranes conformational changes could be enough to alter cell function. We do not know whether peripheral nerve membranes are subject to such
changes. DS500 generally increases cell pinocytosis and phagocytosis (Cohn & Parks, 1967; Ginsberg et al., 1981) and can act as both an adjuvant or a suppressor in cell-mediated immune responses (Diamantstein et al., 1971; Bradfield et al., 1974; McCarthy et al., 1977; Babcock & McCarthy, 1977).

B lymphocyte populations are activated polyclonally by DS500 at an early stage in their differentiation (Vogt et al., 1973; Dörries et al., 1974; Gronowicz et al., 1974). LPS is also a polyclonal B cell activator and mitogenic in mice (Andersson et al., 1972; Gronowicz & Coutinho, 1974; Gronowicz et al., 1974) but shortens peripheral scrapie pathogenesis by increasing the effective titre (Dickinson et al., 1978; C. F. Farquhar, unpublished). Their opposing effects might be due to their acting on B cell populations of different maturity or indeed on completely different B cell subsets (see Gronowicz & Coutinho, 1976). However, direct B cell involvement seems unlikely in the initial stages of uptake and processing of the inoculum given the general lack of humoral responses to early, or any stage of, infection (Chandler, 1959; Gardiner, 1965; Clarke & Haig, 1966; Porter et al., 1973). The only evidence so far for any direct B cell activity in scrapie pathogenesis has come from those models where scrapie infection subsequently alters the relative proportions of IgG isotypes (Collis, 1984) and one mouse model where there is a transient suppression of polyclonal B cell activation (Garfin et al., 1978).

T cell-mediated responses are also affected by DS500 (Bradfield et al., 1974; Vachek & Kolsch, 1975; McCarthy et al., 1977; L'Age-Stehr & Diamantstein, 1977; Babcock & McCarthy, 1977). It seems unlikely that these cells figure prominently in the primary stages of infection by altering infectivity or infectibility, especially in view of the fact that thymectomy has no effect on scrapie pathogenesis (Fraser & Dickinson, 1978). Certainly PHA, a murine T cell mitogen, induces increased susceptibility to peripheral scrapie infection but the initial events, once altered, are set over a very short time scale, a few hours prior to scrapie injection (Dickinson et al., 1978). No such restriction appears to operate with DS500.

Scrapie infectivity has been reported to associate with macrophages (Lavelle et al., 1972; Carp & Callahan, 1981; Marsh, 1981) and certainly they are likely candidates for a role in uptake, degradation and processing of the inoculum, and possibly in the early cell-to-cell transmission of infectivity when titres are increasing in lymphoreticular sites such as the spleen. This is particularly relevant because in the crude inocula used the infective units are closely associated with the tissue debris. However, incubation of macrophages having different Sinc genotypes with 5000 g supernatants of scrapie mouse brain (strains 22A and 22C; A. G. Dickinson & C. F. Farquhar, unpublished) indicates that they may only have a passive role (at least in vitro) as there was no evidence of replication, and infectivity slowly declined over a period of 2 to 3 weeks. Marsh (1981) also reported that adherent peritoneal cells do not retain scrapie infectivity beyond the 3 weeks after infection, either in vivo or in vitro. Given the length of time that DS500 can be sequestered in macrophages (Ehlers et al., 1984) the possibility cannot be ruled out that their interaction in vivo with agent is altered in such a way as to enable them to reduce susceptibility to scrapie. Some such mechanism could explain the unusual, protracted aspects of the DS500 effect. Certainly, although DS500 is traditionally considered to blockade the reticuloendothelial system, at the doses used in this work it is not cytotoxic for tissue macrophages, and while some aspects of function such as phagolysosome fusion are interrupted (Bloksma et al., 1980), this temporary impairment by DS500 normally does not alter responses dependent on antigen presentation (Diamantstein et al., 1971). Indeed, the measurement of such immunological parameters at points throughout infection may well fail to uncover long-term interactions between cells and these 'unusual' agents. Silica, which also induces a reticuloendothelial blockad but is widely toxic for macrophages (Allison et al., 1966), has no effect on peripheral scrapie pathogenesis (Ehlers et al., 1984; R. H. Kimberlin & C. A. Walker, unpublished).

After peripheral infection, the agent quickly disappears from the blood and in its role as an organ of clearance the spleen is closely involved in this process. Scrapie titre increases earliest in the spleen and in the lymph nodes irrespective of the injection route (Fraser & Dickinson, 1970, 1978; Kimberlin & Walker, 1979a). DS500 profoundly alters leukocyte trafficking and
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localization, mobilizing lymphocytes from the spleen and the lymph nodes while delaying their entrance into the latter organs at the capillary high endothelial venules (Sasaki & Suchi, 1967; Bradfield & Born, 1974; Freitas & de Sousa, 1977; Ford et al., 1978). The alteration of the dynamics of the normal cellular microenvironment of these organs (by specific cell egress and an interruption in replacement) may be sufficient in terms of agent interaction to alter scrapie incubation period and titre.

Whatever the interactions between scrapie, DS500 and lymphoreticular cells in peripheral scrapie pathogenesis when scrapie and the modulatory treatment are given relatively close together, it is difficult to postulate a satisfactory mechanism when scrapie infection has been established for 1 or 2 months before DS500 administration. The situation is at present further clouded by the dichotomy in the results between almost half of the animals where no delay was produced and the rest where delay was at least equal to that obtained in other experiments where DS500 treatment occurred much more closely after scrapie injection. It is interesting to speculate on the possibility of a crucially timed switch mechanism in some processing event prior to invasion of the CNS. At this stage in the infectious process, the titre in the spleen has reached a plateau; it is not certain whether this plateau is due to the exhaustion of new replication sites or is a result of the establishment of a dynamic equilibrium between replication and degradation. Prolongation of incubation by interference at this relatively advanced stage suggests either an increase in any degradation or an alteration in the timing or efficiency of spread to the CNS. This may well not be governed by interactions between DS500 and lymphoid cells but between DS500 and peripheral nerves (Kimberlin & Walker, 1979a). Alternatively, these delays could operate within the CNS where the evidence for spread along nerves is becoming more firmly established (Kimberlin & Walker, 1982). This is an obvious area for further investigation.

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