THE EFFECT OF POST-INFECTION IMMUNIZATION ON THE SEVERITY OF EXPERIMENTAL VISNA

By

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INTRODUCTION

Visna is a chronic progressive disease of the central nervous system of sheep caused by an exogenous retrovirus. The early lesions in the nervous system are mainly inflammatory in nature (Georgsson, Palsson, Panitch, Nathanson and Petursson, 1977; Petursson, Nathanson, Georgsson, Panitch and Palsson, 1976). Focal demyelinating lesions of the brain and spinal cord may occur at irregular intervals in some animals and those of the spinal cord are associated with progressive paralysis of the limbs (Georgsson, Nathanson, Palsson and Petursson, 1976; Sigurdsson, Palsson and Grimsson, 1957; Sigurdsson, Palsson and van Bogaert, 1962).

We have postulated (Petursson, Nathanson, Palsson, Martin and Georgsson, 1978) that the early inflammatory lesions, which consist mainly of lymphocytes, macrophages, and plasma cells, are immunologically mediated. Nathanson, Panitch, Palsson, Petursson and Georgsson (1976) showed that a vigorous immunosuppressive regime would almost totally suppress those lesions which developed within 1 month of infection. Since the frequency of virus isolations in anti-thymocyte serum-treated sheep was similar to that in infected controls, and since there was no evidence of a direct virus-induced cytopathology (either cellular destruction or syncytium production), it appeared likely that the early lesions were immune-mediated.

It was deemed desirable to test further the immunological hypothesis and because transfer of immune cells was not possible in outbred sheep, it was proposed to examine the effect of immunization. Immunization prior to infection might suppress virus replication and was to be avoided. Another strategy was adopted which exploited the slow nature of visna infection. Sheep were first infected and were held for an interval to permit infection to become established and the animals were then immunized in an attempt to exacerbate the severity of lesions.

This paper reports the results of such immunization experiments. Additional experiments, to determine the influence of virus dose, are also included be-
cause the results are considered to be relevant to the interpretation of the immunization studies.

MATERIALS AND METHODS

Animals. Male or female Icelandic sheep 6 to 12 months old were used (Petursson, et al., 1976). These sheep were known to be uninfected since naturally occurring visna has been eradicated from Iceland (Petursson et al., 1976, 1978).

Virus. The 1514 strain of visna virus was used. Sheep were infected by intracerebral inoculation of 0.3 to 0.4 ml, containing about 10⁸ TCD50 per inoculum. Virus was propagated and titrated in primary sheep choroid plexus cultures and infected sheep tissues were tested for virus by use of homogenates, explants or explants plus co-cultivation. These techniques have been described earlier (Petursson et al., 1976).

Histology. The severity of CNS lesions was determined by a standardized examination of a stereotyped series of sections of the brain and spinal cord, according to a protocol described earlier (Petursson et al., 1976). An overall grade of 0 (normal) to 6 (most severe visna lesions) was assigned to each animal.

Sero logical tests. Neutralization (N) and complement fixation (CF) tests have been described previously (Petursson et al., 1976).

Immunogens. Two immunizing preparations were used.

(a) Infected autologous cells were prepared by castrating male lambs when 6 months old and preparing primary testis cultures, by the same culture technique employed for primary choroid plexus cells (Petursson et al., 1976). A set of ten 150-ml flasks, with confluent cells, was infected with 10⁸ TCD50 per flask. When a marked cytopathic effect had developed (about 5 days), the cultures were scraped off and were collected in a total of about 50 ml of medium without serum. This harvest was concentrated by lyophilization and reconstituted to 5 ml. For each immunization, 2.5 ml of antigen was homogenized with 2.5 ml of Freund's complete adjuvant (FCA) and a total dose of 5 ml injected in multiple intradermal and intramuscular sites. Each sheep was immunized with infected autologous cells, and controls received uninfected autologous cells.

(b) Visna virions were prepared by a method similar to that described by Haase and Baringer (1974) with final purification by centrifugation through a continuous sucrose gradient. The virus banded at 1:15 to 1:17 g per ml and the harvested band had a protein concentration of about 0.4 mg per ml, and an infectious titre of about 10⁸ TCD50 per ml, 100-times the initial virus harvest. Each immunization consisted of 2.5 ml of virus (freshly treated with 0.25 ml of 1 per cent NP40) plus 2.5 ml of FCA, and the 5 ml of emulsion was injected into multiple intradermal and intramuscular sites. A sham inoculum for control sheep consisted of sucrose in buffer at a density of 1.16 g per ml.

Potency of immunogens. To test the immunogenicity of the antigen preparations, they were inoculated into Wistar rats in which visna virus does not replicate. Animals were injected at day 0 in 4 footpads (total 0.25 ml antigen plus 0.25 alum). Rats were bled 14 days after the second immunization and were tested by CF. The visna virion preparation was immunogenic since 8 of 10 rats developed antibody with a median titre of 36. Infected cells, however, were not immunogenic in this test since only 1 of 10 rats had detectible antibody at the screening dilution of 9.

Immunization schedule. Sheep were infected by intracerebral inoculation and immunized twice, 3 and 5 weeks after infection. Animals were bled weekly for serum, and cerebrospinal fluid was obtained at 4 and 8 weeks. Sheep were killed at 8 to 9 weeks and a standard set of CNS and other tissues were tested for virus and examined for lesions.

Virus dose experiments. To compare a high virus dose with a lower one, a concentrated inoculum was made by centrifugation of a standard 1514 stock at 100 000 g for 1 h in a SW27.1 rotor. The pellet was used as the high dose and a 1000-fold dilution as
the lower dose. Sheep were killed about 1 month after infection, tissues tested for virus, and the CNS lesions graded for severity.

RESULTS

Immunization

Two antigen preparations were used in different experiments. One preparation consisted of infected cells, selected because this immunogen could mimic the target antigen in natural infection. To avoid unwanted allogeneic effects, it was considered important to use autologous cells. To accomplish this, male lambs were castrated, testis cell cultures grown and infected, and a crude harvest used as antigen. Each animal then received antigens prepared from its own cells, and controls were likewise immunized with uninfected autologous cells.

The other antigen consisted of gradient-purified virions, which were disrupted with a non-ionic detergent (NP 40). This preparation was selected to present a high concentration of all virus-encoded structural antigens, relatively free of self (sheep) antigens.

Immunization with Infected Autologous Cells. Seven infected sheep were immunized with infected autologous cells and were compared with 6 infected sheep immunized with uninfected autologous cells. The results (Table 1) failed to show any enhancing effect of immunization. Weekly serological tests showed that each group raised a CF response at essentially the same rate with antibody first detected at 5 weeks (range 3 to 6 weeks). Thus, it is unclear whether the failure to produce an effect was due to the low potency of the immunogen or to an absence of the relevant viral antigens in the immunizing inoculum. Unfortunately, it was not practicable to prepare an immunogen of greatly increased mass, so this approach was abandoned.

<table>
<thead>
<tr>
<th>Immunized with</th>
<th>CNS lesion grades</th>
<th>Median grade</th>
<th>CF Titre at 6 weeks after infection</th>
<th>Virus isolations from the CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected autologous cells</td>
<td>0, 0, 0, 0, 2, 3, 4</td>
<td>0</td>
<td>64–512 (64)</td>
<td>5/42 (5 per cent)</td>
</tr>
<tr>
<td>Uninfected autologous cells</td>
<td>0, 0, 0, 3, 3, 4</td>
<td>1.5</td>
<td>8–32 (16)</td>
<td>6/36 (17 per cent)</td>
</tr>
</tbody>
</table>

* Male lambs were castrated and testis cultures (infected or uninfected) were used to prepare immunogens. Animals were immunized at 3 and 5 weeks after infection and killed at 8 weeks. Each lesion grade represents an individual sheep.

Immunization with Purified Virions. A group of 8 sheep was infected intracerebrally, immunized with purified virions in FCA at 3 and 5 weeks, and killed at 9 weeks. They were compared with 8 sheep sham-immunized with fluid from a control gradient. These results are shown in Table 2. In this experiment the CF test showed a clear effect of immunization, since antibody appeared
at 5 weeks (range 4 to 6 weeks) in immunized sheep, but not until 7 weeks in any control animals.

**Table 2**

**Effect of Immunization with Purified Virions upon the Severity of CNS Lesions in Icelandic Sheep Infected Intracerebrally with** 10⁶ TCD₅₀ **of Strain 1514 of Visna Virus**

<table>
<thead>
<tr>
<th>Immunized with</th>
<th>CNS lesion grades (median)</th>
<th>Median grade</th>
<th>CF Titre at 6 weeks after infection (median)</th>
<th>Virus isolations from the CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>4, 4, 4, 3, 2, 2, 1†</td>
<td>3-5</td>
<td>32–512 (36)</td>
<td>16/48 (33 per cent)</td>
</tr>
<tr>
<td>Control fluid</td>
<td>5, 3, 2, 1, 0, 0, 0, 0</td>
<td>0-5</td>
<td>&lt;8 (9/48)</td>
<td>19 per cent</td>
</tr>
</tbody>
</table>

* Female lambs were immunized with purified virus in FCA at 3 and 5 weeks after infection and were killed at 9 weeks. Each lesion grade represents an individual animal.
† P ≈ 0-1, by a binomial calculation.

Immunized sheep had more severe lesions than control animals, with median scores of 3-5 and 0-5, respectively. However, the differences are of borderline statistical significance because of the small numbers involved.

**Virus Dose**

To determine whether there was a correlation between the extent of CNS virus infection and the severity of lesions, sheep were infected with two different virus inocula, a high dose (10⁸-4 or 10⁸-6 TCD50) and a dose (10⁴-9 or 10⁵-6 TCD50) about 1000-times lower. In view of the slow replication of visna virus, it was predicted that there might be a correlation between inoculum size and extent of infection at slaughter 1 month later. The results are shown in Table 3.

**Table 3**

**Effect of Intracerebral Dose of Visna Virus upon Severity of CNS Lesions and upon Virus Isolations in Icelandic Sheep Inoculated Intracerebrally with Strain 1514 and Killed 1 Month Later**

<table>
<thead>
<tr>
<th>Log 10 virus dose per sheep</th>
<th>CNS lesion grade</th>
<th>Median grade</th>
<th>Virus isolations from the CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4-8-6</td>
<td>4, 4, 3, 3, 3, 3, 2</td>
<td>3</td>
<td>22/48 (46 per cent)</td>
</tr>
<tr>
<td>4-9-5-6</td>
<td>4, 2, 1, 1, 1, 1, 0, 0</td>
<td>1</td>
<td>14/48 (29 per cent)</td>
</tr>
</tbody>
</table>

* Two separate experiments were done and doses varied slightly. Isolations refer to choroid plexus, medulla, cerebellum, and cervical, thoracic, and lumbar spinal cord.

There was a definite correlation between inoculum size, lesion severity and frequency of virus isolation. The high dose sheep had a median lesion grade of 3, with isolations from 46 per cent of CNS specimens, while the low dose animals had a median lesion grade of 1 with isolations from 29 per cent of CNS specimens.

The same data were examined in another way by grouping sheep according to lesion grade regardless of size of inoculum. As shown in Table 4, animals
with the highest lesion scores and highest CSF cell counts yielded the greatest number of isolations.

**Table 4**

<table>
<thead>
<tr>
<th>CNS lesion grade</th>
<th>Number of sheep</th>
<th>CSF cell count &gt; 100 per µl</th>
<th>Virus isolations from the CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 or 4</td>
<td>8</td>
<td>6/8 (75 per cent)</td>
<td>24/48 (50 per cent)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1/2 (50 per cent)</td>
<td>4/12 (33 per cent)</td>
</tr>
<tr>
<td>0 or 1</td>
<td>6</td>
<td>1/5* (20 per cent)</td>
<td>8/36 (22 per cent)</td>
</tr>
</tbody>
</table>

* Same experiments as those summarized in Table 3. CSF tested at slaughter. Cell count was not done on 1 animal.

**Discussion**

**Immunization and Visna Lesions**

The pathogenesis of visna clearly needs further study, but the cumbersome nature of the model excludes many of the experiments which have been done with other viruses, such as lymphocytic choriomeningitis of mice. One key question in visna is whether the lesions are immune-mediated, an hypothesis suggested by the features of the early pathology and by our prior immunosuppression experiments. Further experimental evidence would be useful and the immunization studies were therefore undertaken.

There are several premises which underlie the immunization experiments and these should be examined.

**Measure of Immune Response.** In order to judge the adequacy of immunization, it was essential to measure the response. Unfortunately, we have not been able to develop a reliable assay for the cellular immune response, since lymphocyte blast transformation (Griffin, Narayan and Adams, 1978; Petursson et al., 1978) has given inconsistent results. We were therefore forced to depend upon serological tests.

**Potency of Immunogens.** Concentrated purified virions, when incorporated into FCA, appeared immunogenic as judged by the accelerated serological response in immunized rats and sheep. However, the inoculum of infected autologous cells was probably inadequate, as suggested by the absence of a response in rats or sheep.

**Experimental Schedule.** Several rather arbitrary decisions were made in the hope of maximizing the possible effect of hyperimmunization. Immunization was delayed to 3 weeks after infection, to minimize a possible protective effect. Since virus isolations were not reduced in the immunized animals, this unwanted effect appears to have been avoided. To maximize a possible immuno-enhancement, sheep were examined 3 weeks after the second hyperimmunization, but this represented only a guess as to optimal timing.

Undoubtedly, if it had been possible to conduct multiple experiments in which the foregoing parameters were varied, an enhancing effect might have been seen more clearly. Practical constraints, however, compelled us to accept...
results which were consistent with the immunopathological hypothesis but which fell short of definitive evidence.

**Antigen Target and Lesion Severity**

In the immunization experiments, another implicit assumption was that the extent of the immune response was a parameter which determined lesion severity. However, this may not be entirely correct, since other parameters may play a more critical limiting role.

Specifically, in this slow infection, the extent of the antigen target could be the key constraining variable. It is well documented in classical models of the delayed type hypersensitivity response (Silverstein and Borek, 1966) and in arenavirus immunopathology (Borden and Nathanson, 1974; Gilden, Cole, Monjan and Nathanson, 1972; Nathanson, Monjan, Panitch, Johnson, Petursson and Cole, 1975) that the extent of the antigen target determines the extent and severity of lesions. Visna replicates to a minimal extent in ovine tissues and we have repeatedly seen (Georgsson, Petursson, Palsson, Miller and Nathanson, 1978; Palsson, Georgsson, Petursson and Nathanson, 1977; Petursson et al., 1976, 1978) that there is a strong correlation between virus isolation frequency and lesion severity.

To explore the role of antigen target size, a comparison was made of two virus doses, and the larger dose was associated with more severe lesions. Furthermore, when individual animals were compared, lesions were again correlated with virus isolation frequency.

These observations suggest that antigen target is an important determinant in severity. If this is so, it may be that enhancing the immune response can only exert a minimal effect. In conclusion, the data are compatible with the view that an antiviral immune response is necessary but is not by itself sufficient for the production of early visna lesions.

**SUMMARY**

Visna is a slow retrovirus infection of sheep which produces both inflammatory and demyelinating lesions of the central nervous system. A prior study indicated that immunosuppression markedly reduced the early inflammatory lesions.

To further explore immunological determinants in the pathogenesis of visna, infected sheep were immunized 3 and 5 weeks after intracerebral virus infections and killed at 8 to 9 weeks. When infected autologous cells in Freund’s complete adjuvant (FCA) were used as immunogen, there was no evidence of an accelerated immune response and no effect on lesion severity. When purified concentrated virus in FCA was used as immunogen, there was an accelerated immune response and a modest increase in lesion severity.

Because visna virus replication is very slow in vivo, it was hypothesized that the extent of the antigen target might be a limiting parameter in disease progression. Comparison of 2 virus inocula, $10^8$ and $10^9$ TCD50, indicated that the larger dose was associated with more severe lesions. This is consistent with
the view that the size of antigen target limits lesion severity, and could explain why immunization has only a modest influence on the extent of CNS pathology.

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REFERENCES


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