Introduction: Visna in Iceland

Iceland has played a special role in the history of visna, since the disease was initially recognized in Iceland and carries the name (visna is the Icelandic word for wasting) used by Sigurdsson (1954) to describe one of the presenting signs of the disease. The following brief account is designed to complement the detailed review by Narayan and will be focused on studies conducted in Icelandic sheep.

Visna virus was originally inadvertently introduced into the Icelandic sheep population, which was apparently free of the infection, as a consequence of the importation from Germany of 20 karakul rams in 1933. The infection spread through the northern and western regions of Iceland and was recognized as an epizootic in the early 1940s. Bjorn Sigurdsson, an Icelandic physician, showed that the disease was due to a transmissible agent, and he and his collaborators isolated the virus by growing it in cultures of sheep choroid plexus (SCP) cells. A heroic effort by veterinarians and farmers, during the period 1944–1965, led to the eradication of visna, so that Icelandic sheep are now free of infection. These events are described in a classical series of publications (Sigurdsson, 1954; Sigurdsson et al., 1957, 1958, 1960, 1962; Gudnadottir, 1974; Palsson, 1976).

More recently, we have conducted a collaborative study of the pathogenesis of visna in Icelandic sheep (Petursson et al., 1976, 1978, 1979, 1981; Georgsson et al., 1976, 1977, 1980, 1982; Nathanson et al., 1976, 1979; Palsson et al., 1977; Martin et al., 1982; Lutley et al., 1983). The following account will summarize selected aspects of these studies, with particular emphasis on the pathogenesis of the lesions seen in the
central nervous system (CNS) and on the phenomenon of antigenic drift.

Biological Variation in the Virus and in the Host

As with other viruses, genetic determinants in virus and host have a marked influence on visna infection and its pathological consequences. This accounts in great part for differences in experimental ovine infections with visna, maedi, or progressive pneumonia viruses, reported by different laboratories. Studies in Iceland have utilized exclusively virus strains which are highly cytolytic in SCP cells; such viruses grow to high titer in cell culture and tend to kill infected cells in contrast to field isolates of progressive pneumonia virus (Narayan et al., 1982) which produce syncytia with release of only low titers of cell-free virus. Furthermore, we have used virus strains that have been selected for their ability to cause severe CNS disease. The second important variable is the high susceptibility of Icelandic sheep. Compared to certain English breeds found in the United States, these animals are more prone to clinical disease and, in vivo, are more permissive for visnavirus.

Virus Replication and Immune Response in Icelandic Sheep

Virus Replication

Following intracerebral inoculation of a large dose (10^6 TCD50) of visna virus in Icelandic sheep, some animals were tested repeatedly for virus in blood and spinal fluid, while others were sacrificed at intervals and many tissues examined for virus. Salient observations were as follows.

(i) Viremia appeared at 2–4 weeks and persisted throughout the life-long infection (Petursson et al., 1976). Virus was isolated from buffy coat, but never from plasma, even before the appearance of neutralizing antibody in the serum. Isolations required co-cultivation of leukocytes with permissive SCP cells, and cultures often yielded virus only after 2–4 weeks of incubation. Maximal frequency of isolations required the use of 10^6 leukocytes, and fewer isolations were made when 10^5 or 10^4 leukocytes were tested. Although almost all sheep were viremic, only 10–80% of serial specimens from individual sheep were positive.

These observations suggest that virus was carried as a latent provirus in a small number of leukocytes. Consistent with this, extensive immuno-fluorescent or electron microscopic examination failed to identify individual virogenic cells. Testing of fractionated cells suggests that
mononuclear cells, probably both lymphocytes and monocytes, can carry the genome (Petursson et al., 1976; Narayan et al., 1982).

(ii) Virus can be isolated from other tissues (Petursson et al., 1976), mainly from lymphoid organs, central nervous system and lung, throughout the life of the infected sheep. Titration of tissue homogenates indicates that minimal amounts of cell-free infectious virus are produced, and many specimens require explantation and co-cultivation to isolate virus (Georgsson et al., 1978; Petursson et al., 1976).

(iii) Cerebrospinal fluid (CSF) yields low titers of cell-free virus, but only for the first 3 months after infection. With the appearance of neutralizing antibody in the CSF, virus can no longer be isolated (Nathanson et al., 1979).

**Immune Response**

Visna virus is immunogenic and infected Icelandic sheep consistently raise an immune response.

(i) Serum neutralizing antibody appears 2–3 months after infection (Petursson et al., 1976). Immunodiffusion tests show that all infected Icelandic sheep generate serum antibody against the viral glycoprotein (neutralizing antigen) and that about half also make detectable antibody against the p30, the major core antigen (Georgsson et al., 1980).

(ii) CSF neutralizing antibody appears after serum antibody, in about half the infected sheep (Nathanson et al., 1979) and CSF/serum antibody ratios are as high as 1:1 to 1:10. Since the blood–brain barrier remains intact, this CSF antibody represents intrathecal synthesis, by B lymphocytes which migrate into the CNS. Plasma cells are regularly seen in the inflammatory lesions in brain and spinal cord (Georgsson et al., 1977).

Measurement of total immunoglobulin in CSF indicates that IgM is elevated in many sheep, but IgG levels are not. Electrophoresis of spinal fluid in long-term infected sheep shows oligoclonal bands of immunoglobulin in about half the animals.

(iii) Cell-mediated immunity has been assessed by lymphocyte blast transformation in response to stimulation by virus or virus-infected fibroblasts. Although not readily detected in Icelandic sheep, studies by other investigators (Griffin et al., 1978; Siivonen, 1981; Larsen et al., 1982) indicate that a response can be seen in peripheral blood and CSF cells, peaking at 2–8 weeks following infection, and then waning, with irregular reappearances.
Slowness and Persistence

These observations are consistent with the view that in vivo sheep cells restrict the expression of the viral genome. Direct evidence of this interpretation has been generated by the painstaking studies of Haase and Brahic (Haase et al., 1977; Brahic et al., 1981), who have applied in situ hybridization to infected sheep tissues. This restriction is the salient molecular aspect of visna infection and undoubtedly contributes to the slowness of infection. Another factor contributing to slowness is the damping effect of neutralizing antibody in serum and in extracellular fluid of the CNS.

Persistence of the viral genome in the face of a potent immune response is explicable by the same restriction phenomenon, which perpetuates the viral genome as a provirus in cells that do not synthesize viral antigens. Such latently infected cells can escape immune surveillance. Presumably, an occasional cell becomes virogenic, contributing to the indolent spread of the infection. The precise controls on retrovirus gene expression are not yet well understood (Varmus, 1982).

Early and Late Visna Lesions in Icelandic Sheep

All Icelandic sheep inoculated intracerebrally with a large dose (10⁶ TCD₅₀) of a neurotropic strain (1514) of visna virus develop lifelong infections, as signalled by the ability to isolate virus from the blood and by persistent serum antibodies. Two types of pathological changes occur in the CNS of such sheep: (i) an early and persistent inflammatory response, seen in most sheep; and (ii) the irregular and unpredictable occurrence of focal demyelinating lesions which, in the spinal cord, produce clinical paresis. For clarity these two lesions will be described separately, followed by a discussion of mechanisms.

Early Inflammatory Lesions

The majority of Icelandic sheep infected by intracerebral injection of the 1514 strain of visna virus develop inflammatory CNS lesions as described in several publications (Georgsson et al., 1976, 1977, 1979, 1982; Petursson et al., 1976). To summarize:

(i) CNS lesions are distributed mainly around the ventricles, although lesions may be seen in white matter and meninges. These lesions consist of accumulations of lymphocytes, monocytes or macrophages, and plasma cells, distributed as perivascular cuffs, or as focal and diffuse infiltrates of
the neuropil. There is little evidence of neuronal death, or neurophagia, and overt demyelination is also unusual. Occasional areas of severe inflammation may contain foci of necrosis or liquefaction, but otherwise there is little tissue destruction.

(ii) CSF examination permits the serial description of the inflammatory process in individual sheep (Nathanson et al., 1979). The course of inflammation clearly differs in individual sheep, but the most common pattern is an early rise in cells, at 0.5–3 months after infection, with a subsequent waning of pleocytosis, which may persist at slightly elevated levels or drop to normal limits. The great majority of CSF cells are lymphocytes or macrophages. There is a general correlation between cell count and severity of inflammation, and the onset of clinical signs is often accompanied by CSF pleocytosis.

(iii) These inflammatory changes are usually subclinical.

Clinical Signs and Late Demyelinating Lesions

Visna was originally described (Sigurdsson et al., 1957, 1958) as a demyelinating disease, but the observations (Sigurdsson et al., 1962) were ambiguous in the absence of ultrastructural studies. The uncommon and unpredictable occurrence of clinical disease and the difficulties of perfusion impeded recent work, but it has finally been possible to confirm primary demyelination as an important component of clinical disease (Georgsson et al., 1982).

(i) Clinical signs are characteristically irregular and unpredictable in Icelandic sheep, with onset at any time from 3 months to as late as 8 years, as reflected in Fig. 1. The salient feature is lameness, usually in one or both hindlegs, with progression to all four extremities. The course of paresis may be rapid, over several weeks to months, or may be gradual and irregular, over several years. Eventually sheep cannot feed themselves and must be sacrificed.

(ii) Sheep sacrificed because of progressive lameness usually show focal demyelinating lesions in the spinal cord, in addition to subacute encephalomyelitis. These focus lesions are often dramatic when viewed at low power (Fig. 2), because of marked loss of myelin and rather sharply demarcated borders. Within these foci, electron microscopy (Fig. 3) shows classical primary demyelination, with many naked but otherwise intact axons. Remyelination, by oligodendrocytes or by Schwann cells, is also prominent. Within these chronic demyelinated foci, inflammation is minimal and gliosis is marked. In more recent demyelinated lesions, debris-laden macrophages and inflammation are more prominent.
Mechanisms of CNS Lesions

As an experimental model visna is cumbersome, because of the slow and variable time course, the size and expense of the animals, and the absence of inbred histocompatible sheep. The following discussion necessarily falls back on speculation to fill gaps in the evidence.

(i) The lesions of visna, particularly the late lesions, are excessive in relation to the minimal numbers of infected cells. Thus it is necessary to invoke an amplifying step to explain the severity of pathology. An immune mechanism would provide this amplification step.

(ii) Inflammatory lesions evolve during the first 2–4 weeks after infection and seem to stabilize after 3 months. The evolution of the blast transformation response (Griffin et al., 1978), which peaks between 3–6 weeks, is congruent with this tempo, while the antibody response in serum and CSF is somewhat slower, reaching peak at 3–6 months after infection.

(iii) Suppression of the immune response (Nathanson et al., 1976) markedly reduces the early inflammatory lesions without reducing virus isolations from the CNS (Table 1). Conversely, post-infection immunization (Nathanson et al., 1981) enhances lesions, although not markedly.
(iv) The available evidence suggests that the target antigen is viral and not myelin. There is a definite correlation between severity of subacute encephalitis and the frequency of viral isolation (Petursson et al., 1976; Georgsson et al., 1978; Nathanson et al., 1981), as shown in Table 2. Also, the dose of infecting virus correlates with lesion grade.

Experimental autoimmune encephalomyelitis (EAE) can be induced in sheep immunized with sheep brain in CFA, and sensitized animals raise antibodies against sheep basic protein and galactocerebroside. However, asymptomatic visna-infected sheep fail to raise serum antibodies against either of these myelin antigens up to 9 months after infection (Panitch et al.,
Fig. 3  Electron micrograph of demyelinated axons in the spinal cord of sheep 1527, sacrificed with clinical signs 6.5 years after infection. Two well-preserved demyelinated axons are shown, with adjacent astrocytic processes. Magnification × 24000.
Table 1  Influence of immunosuppression upon severity of early visna lesionsa

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood lymphocytes</th>
<th>CSF cells</th>
<th>CNS lesion grade</th>
<th>CNS virus isolations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppressed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(8 sheep)</td>
<td>Median</td>
<td>25b</td>
<td>17b</td>
<td>00</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0–180</td>
<td>11–268</td>
<td>0–2</td>
</tr>
<tr>
<td>Control</td>
<td>Median</td>
<td>5000</td>
<td>246</td>
<td>3</td>
</tr>
<tr>
<td>(8 sheep)</td>
<td>Range</td>
<td>4000–7400</td>
<td>15–1500</td>
<td>1–4</td>
</tr>
</tbody>
</table>

*aAfter Nathanson et al. (1976). Icelandic sheep were treated with horse anti-sheep thymocyte serum, or normal horse serum, infected with $10^6$ TCD50 of 1514 strain of visnavirus, and sacrificed 1 month later, when all determinations were made.

bCell count per microliter.

cInflammatory lesions graded on a scale of 0–6 for each animal. Only 1/8 suppressed sheep had lesions compared to 8/8 controls.

dPercentage of isolations based on four or five samples tested on each animal.

Table 2  Correlation between severity of early visna virus lesions and the frequency of virus isolations

<table>
<thead>
<tr>
<th>CNS lesion grade</th>
<th>No. of sheep</th>
<th>Number (% with CSF cell counts over 100)</th>
<th>Number (% of CNS virus isolation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>17</td>
<td>3/15 (20)</td>
<td>17/80</td>
</tr>
<tr>
<td>2+</td>
<td>35</td>
<td>18/32 (56)</td>
<td>88/148 (59)</td>
</tr>
</tbody>
</table>

*aComposite data, after Petursson et al. (1976) and Nathanson et al. (1981).

bCounts per microliter, at sacrifice.

cPercentage of isolations based on four or five specimens tested on each animal.

1976). Finally, the ability of visna–maedi virus to induce progressive pneumonia, the pulmonary counterpart of subacute encephalitis, is most readily explained by an anti-viral immune mechanism.

The focal demyelinating (or late) lesions of visna, which resemble the plaques of multiple sclerosis, are still poorly understood. We do not know if these are more severe manifestations of the same pathological process responsible for subacute encephalitis. Also, the trigger that initiates the irregular occurrence of demyelination at long intervals after infection is obscure. Demyelination could be mediated as a bystander effect, secondary to a delayed-type hypersensitivity response.
Antigenic Variation During Long-term Infection of Iceland Sheep

Gudnadottir (1974) first reported that a virus isolate from a long-term infected Icelandic sheep could escape neutralization by the animal’s own sera, even though the same sera neutralized the infecting strain. Narayan and colleagues (Narayan et al., 1977a, 1978) further studied this phenomenon in Hampshire and Border Leicester sheep. They found that when blood isolates from each sheep were tested against the animal’s own sera (autologous neutralization), variants were isolated from two of seven animals. It was also shown that variants could be selected in cell culture, by passaging virus in the presence of neutralizing antisera (Narayan et al., 1977a, 1981; Dubois-Dalcq et al., 1979).

Molecular comparison of both the proteins and the genomes of variant viruses (Scott et al., 1979; Clements et al., 1980) indicated that the variants represented point mutations in the env gene coding for the envelope glycoprotein (the protein responsible for neutralization).

Antigenic Drift in Long-term Infected Icelandic Sheep

The occurrence of antigenic variation in some visna-infected sheep is well established. However, several questions still remained unanswered. What is the frequency with which variants occur? Do variants play a role in virus persistence? Do variants play a role in lesion development? A detailed study of antigenic variants was therefore undertaken in our long-term Icelandic sheep (Lutley et al., 1983), and some of the salient observations will be summarized.

A group of 20 Icelandic sheep were infected with strain 1514 and followed over 7 years, during which time 209 isolates were made from blood, CSF and CNS. A stratified sample of 76 of these isolates was selected to represent 19 sheep and differing intervals after infection. These isolates were tested by neutralization against three reference sera, and the results are set forth in Fig. 4. Among the 76 isolates, 12 (16%) were typed as variants. These variants were dispersed over time and among sheep. In only one instance (sheep 1557) did variant viruses replace the infecting strain, and the frequency of variants did not increase with time after infection.

Among the 20 sheep, seven animals were selected (with emphasis on those with clinical visna) and a total of 35 isolates were each tested against serial sera from the same sheep (autologous neutralization). The findings are summarized in Table 3, which shows that only isolates from sheep 1557 evinced drift, while none of 27 isolates from the other six animals demonstrated this phenomenon. Also, none of the CNS isolates from sheep with clinical visna typed as antigenic variants.
Fig. 4 Typing of 76 isolates from blood or CNS of 19 Icelandic sheep inoculated intracerebrally with $10^6$ TCD$_{50}$ of visna virus strain 1514 and followed for 7 years. Typing by neutralization with sera specific for 1514 and for 796 viruses. ○, Blood isolates; ◦, CNS isolates (total 64 isolates); □ or ◇, variant viruses which were not neutralized by antiserum against 1514 virus (12 isolates). After Lutley et al. (1983).

Table 3 Autologous neutralization tests on 35 isolates from seven Icelandic sheep followed for 7 years after intracerebral injection of $10^6$ TCD$_{50}$ of visna virus strain 1514$^a$

<table>
<thead>
<tr>
<th>Sheep no.</th>
<th>Diagnosis</th>
<th>Survival (years)</th>
<th>Variant isolates$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1520</td>
<td>Visna</td>
<td>2.5</td>
<td>0/5</td>
</tr>
<tr>
<td>1523</td>
<td>Visna</td>
<td>7.5</td>
<td>0/1</td>
</tr>
<tr>
<td>1524</td>
<td>Visna</td>
<td>6.5</td>
<td>0/7</td>
</tr>
<tr>
<td>1552</td>
<td>Visna</td>
<td>1.8</td>
<td>0/4</td>
</tr>
<tr>
<td>1553</td>
<td>Visna</td>
<td>7.2</td>
<td>0/1</td>
</tr>
<tr>
<td>1518</td>
<td>Alive</td>
<td>—</td>
<td>0/2</td>
</tr>
<tr>
<td>1557</td>
<td>Other$^c$</td>
<td>6.6</td>
<td>3/5</td>
</tr>
</tbody>
</table>

Totals 3/24 3/11

$^a$After Lutley et al. (1983).

$^b$Each isolate was tested against serial serum samples from the same sheep. Variants were isolates that escaped neutralization by autologous sera.

$^c$Splenomegaly and debilitation.
Biological Significance of Antigenic Variation

In our view, our observations and those of Narayan and colleagues, are quite consistent. An interpretative summary follows.

(i) Antigenic variants of visna virus are constantly generated by spontaneous mutations in the env gene of the virus. This is a manifestation of the plasticity of the genomes of RNA viruses (Holland et al., 1982).

(ii) Persistent infection in the sheep must select somewhat for these variants, since they were found at a frequency over 10%, which is much higher than their frequency in the initial inoculum (presumably less than 1 per 1000 virions, since reference antisera readily neutralize 1000 TCD₃₀ of the 1514 strain).

(iii) In the long-term infected sheep, variants persist together with the parental virus and do not gradually replace the parental serotype. If variants were responsible for continued spread of infection, they should increase in relative frequency but this was not seen. Therefore, antigenic variation does not appear to be essential for virus persistence.

(iv) Isolates from the CNS of animals with clinical visna are usually not variants, i.e. are neutralizable by autologous antisera. Within the limits of these observations, there was no evidence that variants were involved in the occurrence of focal demyelinating lesions.

Summary

A brief review is presented of studies of experimental infection of Icelandic sheep following intracerebral inoculation of a neutropic strain of visna virus. In vivo, replication of the virus is restricted, so that some cells carry the DNA provirus as an unexpressed genome. This cellular restriction plays a major role in the slowness of the infection, and the occurrence of latent provirus assures lifelong persistence of the infection in the presence of neutralizing antibody.

Infected Icelandic sheep undergo two types of CNS disease: (i) a sub-clinical subacute encephalitis begins within weeks of infection in most sheep; and (ii) at irregular intervals from 0.5–8 years after infection clinical paresis develops in 10–90% of Icelandic sheep and is accompanied by discrete focal demyelinating lesions in the spinal cord. The subacute encephalomyelitis is probably mediated by an anti-viral cellular immune response, while the pathogenesis of the focal demyelinating lesions are still obscure.

During persistent infection there is some selection for antigenic variants of the infecting serotype and these are isolated at a frequency of about 15%.
However, variants do not replace the infecting serotype and antigenic drift does not appear essential for virus persistence or for the occurrence of demyelinating lesions.

Acknowledgement

Supported in part by USPHS grant NS 16010.

References


