Visna-Maedi-Like Disease Associated with an Ovine Retrovirus Infection in a Corriedale Sheep

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Abstract. A visna-maedi-like disease was found in a Corriedale sheep from which a retrovirus sharing the group antigen of visna-progressive pneumonia virus was isolated from lung, brain, and spleen. Clinically, the sheep had acute neurologic signs and dyspnea. Pathologic examination showed lesions similar to both visna and maedi. In the lung, there was a patchy interstitial pneumonia with marked lymphoid hyperplasia. Changes in the central nervous system were necrotizing nonsuppurative encephalitis of the brain stem, poliomyelitis of the cervical cord, and ependymitis and subependymal gliosis of the ventricles. Histologically, the central nervous system lesions seemed to have arisen sequentially, perhaps in response to bursts of virus replication as the agent underwent possible antigenic mutation. The severe lesions in both the central nervous system and lungs suggested a virus strain with dual tropism.

Visna and maedi are classic slow viral diseases of sheep, with prolonged incubation periods and slow progressive development of clinical signs [17–22]. Unlike scrapie, another slow viral disease of sheep, visna and maedi are caused by conventional viruses [9]. Visna is a paralytic disease characterized histologically by prominent inflammatory lesions in the subpial or periventricular regions of the brain. The lesions vary from severe necrotizing non-suppurative encephalomyelitis to areas of marked gliosis [22]. In maedi, which is characterized clinically by wasting and dyspnea, the pathologic changes are interstitial pneumonia, marked perivascular and peribroncholar lymphoid hyperplasia with formation of lymphoid follicles, and lymphoid hyperplasia of the mediastinal and bronchial lymph nodes [4, 18].

Widespread outbreaks of maedi occurred among sheep in Iceland after the introduction of Karakul sheep from Germany during the 1930’s [4, 5, 18, 19]. During these epizootics, visna occurred as a rare neurologic complication [5, 22]. Similarly, a maedi-like disease, known as progressive pneumonia in the United States and zwoegerziekte in the Netherlands, occasionally is associated with central nervous system lesions indistinguishable from those of visna in Icelandic sheep [1, 3]. Recent studies have shown that these diseases occurring in different geographic areas are caused by serologically related ovine retroviruses [2, 3, 9]. Moreover, studies in Iceland have shown that the virus isolated from the central nervous system of a sheep
with visna can cause maedi, and maedi virus can cause lesions of visna [6, 7, 17]. Likewise, zwoegerziekte virus can cause lesions of visna in Dutch Texel sheep [3]. Despite these findings, the pathologic potential of various strains of ovine retrovirus has not been evaluated thoroughly. It is not known, for example, whether they have primary tropism for the lung or for the central nervous system or have dual tropism.

**Case History**

A 3½-year-old ewe was one of two Corriedale sheep from a breeding farm in Massachusetts. The ewe was thinner and slower than normal; this was thought to be inconsequential. She became progressively thinner and lethargic and after five months suddenly developed ataxia and circling. Shortly before death, she was unable to rise, and had paddling movements and labored respiration. A lumbar puncture was done and pleocytosis of 76 mononuclear cells/μl was found in the cerebrospinal fluid. The cerebrospinal fluid was bacteriologically sterile. The hematologic and blood chemistry examination showed a mild leukocytosis and elevated serum glutamic-oxaloacetic transaminase (SGOT). Despite intense antibiotic and fluid therapy, the ewe's condition deteriorated during the next two days and she was killed and necropsied. Tissues were fixed in neutral buffered formalin, embedded in paraffin, sectioned at 6 μm, and stained with modified Mayer’s hematoxylin and eosin (HE). Selected sections of brain and lung were stained with luxol fast blue-cresyl violet, Movat’s pentachrome, Masson’s trichrome, Sevier-Munger, and Verhoff-van Gieson stains.

At autopsy, parts of brain stem, lung, and spleen were taken for viral and bacteriological studies. Fragments were minced and explanted in Eagle’s minimum essential medium supplemented with 20% fetal bovine serum and 0.01% gentamicin. Outgrowths from these tissue explants were monitored for development of cytopathic effect. In addition, the ewe's serum was examined for neutralizing antibody to one strain of progressive pneumonia virus (kindly provided by Dr. John Gorham, USDA, Pullman, Washington) and to three strains of visna virus from Iceland (K796, K1010, K1514).

**Results**

At necropsy, the lungs were dry and heavy (1225 grams). They did not collapse, and filled most of the thoracic cavity. The pleural surface was smooth and glistening, but the lungs had patchy areas of gray discoloration, most severe in the anterior ventral area. These gray areas were rubbery on palpation and, as seen on the cut surfaces, extended into the parenchyma. The mediastinal lymph nodes were large, moist, and tan-green.

Microscopically, lesions in the lungs were characterized by large, poorly circumscribed areas of severe interstitial pneumonia with reactive type II pneumocytes and hypertrophy of smooth muscles in interalveolar septa (fig. 1). Moderate epithelial hyperplasia and hypertrophy of the bronchi and bronchioles also were seen. There was a variable interstitial accumulation of lymphocytes, plasma cells, and foamy histiocytes with eosinophilic cytoplasm. Some multinucleated cells were seen in the interstitium; they also occurred in alveolar spaces, as did numerous plugs of neutrophils. These regions of chronic interstitial pneumonia invariably contained multiple prominent foci of lymphoid hyperplasia with frequent formation of lymphoid follicles (fig. 2). Foci often were adjacent to bronchi and bronchioles, although numerous
Fig. 1: Section of lung. Alveolar changes and edge of lymphoid follicle in lower right. Severe interstitial pneumonia with plump alveolar lining cells and smooth muscle hypertrophy. General loss of distal air spaces and several neutrophil plugs in alveoli (arrows). HE. Bar = 100 μm.

Fig. 2: Low-power view of lung. Patchy interstitial pneumonia and numerous lymphoid follicles. Several bronchi have moderate epithelial cell hyperplasia. HE. Bar = 500 μm.
Fig. 3: Section of inflammatory focus in basilar portion of pons with preservation of neurons (arrows), multiple perivascular cuffs, endothelial cell hypertrophy, and moderate astrocytosis. HE. Bar = 100 μm.

Fig. 4: Inflammatory lesion in dorsal horn of cervical cord. Perivascular lymphocytic cuffs, neuronal degeneration (arrows), and gliosis consisting of plump astrocytes. HE. Bar = 100 μm.
perivascular and interstitial foci were seen. Sometimes several foci of lymphoid hyperplasia coalesced and formed larger aggregates. Between the areas of chronic interstitial pneumonia were numerous peribronchiolar, perivascular, and interstitial lymphoid accumulations in otherwise normal lung. In addition to lymphoproliferative changes in the lung, the mediastinal lymph nodes had marked lymphoid hyperplasia and acute lymphadenitis.

Three kinds of lesions were found in the central nervous system: necrotizing nonsuppurative encephalitis of the brain stem, poliomyelitis of the cervical cord, and ependymitis and subependymal gliosis of the paraventricular area.

The most striking lesions in the central nervous system were multiple, poorly demarcated foci of malacia involving the basilar part of the pons and cerebellar peduncles. The malacic foci were most prominent in myelinated regions; there was marked fragmentation and splitting of myelin sheaths. Some foci also extended into surrounding gray matter. Despite the destructiveness of the lesions, however, there was general sparing of neurons and limited preservation of axons. In addition, foci were seen in which the breakdown of myelin was accompanied by marked astrocytosis, microglial proliferation, and extensive perivascular cuffs of mononuclear cells (fig. 3). In other areas, astrocytosis was the only abnormality.

The proliferating astrocytes had an elongated fibroblastic appearance with plump vesicular oblong nuclei and thin linear nucleoli. They were arranged either as interlacing bundles paralleling fiber tracts or as whorls around vessels. The degree of astrocytosis between malacic foci, or even within a single focus, varied, alternating between areas of dense astrocytosis and marked malacia. In the areas of dense astrocytosis, Movat’s pentachrome and Masson’s trichrome stains showed increased amounts of fibrous connective tissue. Verhoff-van Gieson method stained an increased amount of mature collagen around blood vessels.

The microglial reaction consisted of packets of plump macrophages laden with myelin-debris. These cells were found in small numbers between astrocytic bundles or in sheets in the malacic areas. No rod cells were found.

Cells forming the perivascular cuffs consisted of accumulations of lymphocytes, plump astrocytes, and macrophages in varied proportions. Around midsized arteries and veins, the cuffs often were over 10 cells thick. Endothelial cells of medium-sized veins sometimes were hypertrophied.

The second characteristic lesion, poliomyelitis of the cervical spinal cord, was a unilateral circumscribed lesion in the gray matter of the dorsal horn. Neurons in the area had been replaced by many astrocytes. There were large perivascular cuffs composed of mononuclear cells. The reactive astrocytes were primarily gemistocytic, but the perivascular cuffs were predominantly lymphocytic (fig. 4). Sections of the cervical spinal cord also had thickened dorsal meninges caused by fibrosis and moderate lymphocytic infiltration.

The third characteristic lesion was ependymitis and subependymal astrocytosis with the formation of glial nodules. The lateral, third, and fourth ventricles and the
Fig. 5: Choroid plexus and subependymal region of lateral ventricle. Focal ependymitis with loss of ependymal lining cells and mild subependymal gliosis. Poorly circumscribed lymphocytic infiltrate and endothelial cell hypertrophy in choroid plexus. HE. Bar = 100 μm.

periaqueductal tissue of the caudal medulla oblongata were affected. There was little evidence, however, of extension into adjacent gray or white matter. Nodular accumulations of glial cells were most prominent around the lateral ventricle. The choroid plexus in this region also had a moderate lymphocytic and mononuclear cell infiltration (fig. 5).

Other pathologic changes included acute splenitis, generalized acute lymphadenitis, focal acute endocarditis, and focal myocardial degeneration.

Virologic and Immunologic Studies

Cellular outgrowths from explants of brain, lung, and spleen developed polykaryocytes within one month of explantation. Supernatant fluids from these cultures were then inoculated onto monolayers of sheep choroid plexus cells, which are used routinely in our laboratory for cultivating Icelandic visna virus. These cultures developed the type of cytopathic changes already described. Supernatant fluids from the cultures were used as a source of viral antigen for immunodiffusion tests [11] as well as for infectious virus for virus neutralization tests [12].

In immunodiffusion tests, the three viruses obtained from brain, lung and spleen respectively formed a single line of homology with visna virus 1514 when the four
agents were reacted with antiserum to the P25 core antigen of visna virus. This result confirmed previous findings that ovine and caprine retroviruses share P25 antigenic determinants [11, 23]. The three Corriedale viruses, however, are antigenically distinct from the Icelandic visna viruses since the latter agents K796, K1010 and K1514 were neutralized by a hyperimmune serum at dilutions of 1:80, 1:60, and 1:640, respectively. This serum failed to neutralize the Corriedale agents at a dilution of 1:10. Surprisingly, the affected sheep also had no serum neutralizing antibody (1:10) to the three agents isolated from its tissues, or to the visna viruses, or to progressive pneumonia virus. This lack of neutralizing antibody in American sheep with visna–maedi to retroviruses isolated from their tissue is unusual but has been seen in several field cases of this disease complex (Narayan, unpublished). Whatever the mechanism, the lack of neutralizing antibody to the virus in this case study precluded any studies of antigenic comparison.

Discussion

The lesions fulfill the morphologic criteria for maedi and for visna. The pulmonary changes consisted of patchy interstitial pneumonia, marked peribronchiolar and perivascular lymphoid hyperplasia with formation of lymphoid follicles, and lymphoid hyperplasia of the mediastinal and bronchial lymph nodes. These lesions have been described often in sheep affected with progressive pneumonia, maedi, or zwiegerziekte [1, 4, 10, 16]. Since the ovine lung responds to various stimuli with interstitial pneumonia and proliferation of bronchiolar and alveolar epithelium, it was important to distinguish the pulmonary changes in the ewe from other pulmonary conditions, such as lesions caused by lungworms and pulmonary adenomatosis. Except for one focal abscess, there was no gross, histologic, or parasitologic evidence of lungworm infection in the ewe. Although epithelial cell hyperplasia was seen in the most severe areas of interstitial pneumonia, it never simulated the papillary lesion of pulmonary adenomatosis, which has not been described in the United States.

The central nervous system lesions (marked necrotizing nonsuppurative encephalomyelitis, subependymal gliosis, nonsuppurative ependymitis, lymphocytic choroiditis, and lymphocytic meningitis) are all characteristic of the lesions of visna [15, 22]. The differences in age among the lesions of the central nervous system were of interest. Those in the cervical spinal cord, meninges, subependyma, and choroid plexuses appeared older than those in the brain stem. Even the brain stem lesions, however, seemed to consist of acute, subacute, and chronic reactions as indicated by the variability in cellular responses to the malacia. The malacic foci with macrophages were deemed more recent than those with dense astrocytosis and microglial cell proliferation.

Sequential development of lesions in an animal infected with the ovine retroviruses is consistent with their manner of replication: they can replicate from proviral DNA templates in the host cell [8], safely sequestered from immune defenses of the host. Further, although replication of the virus may be followed by development of specific neutralizing antibody, this antibody becomes the driving force for selection of viral
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mutants, which can escape neutralization [5, 12]. Because these mutants are virulent [13], the antigenic drift mechanism theoretically creates a condition for sequential bursts of viral replication and attendant lesions caused by each mutant. Such a mechanism could explain the apparent disparity in the age of the lesions found in brain and spinal cord of the ewe. The lack of neutralizing antibody in the ewe to the viruses obtained could be interpreted to mean that the viruses were antigenically homogeneous and had developed in the animal too recently to have induced formation of neutralizing antibody. Other factors may be involved, however, in the induction of neutralizing antibody to these viruses since inoculation of new Corriedale sheep with this agent has failed to stimulate such antibodies despite persistent infections for more than six months in three animals (Narayan, unpublished).

The sharing of antigenic determinants in the P25 among ovine/caprine retroviruses regardless of pathogenic properties makes diagnosis of infections by these agents relatively simple. The combination and lack of neutralizing antibody in many diseased animals plus antigenic drift of the agent when such antibodies do develop, however, make identification of virus strains in circulation almost impossible. Thus whether multiple strains of virus coexist in sheep and goat herds and whether different strains of virus are endowed with different pathogenic potentials must await development of virus strain specific tests. Until then it may be appropriate to consider visna and maedi a disease complex with characteristic lesions caused by antigenically variable ovine retroviruses rather than the result of infection with specific Icelandic strains of virus.

The pathogenic potential of this group of viruses cannot be evaluated fully without consideration of the breed of animal affected. Prior studies suggest that Icelandic sheep may be more susceptible to visna than English Down breeds of sheep [14, 15]. Recent studies, furthermore, have shown that the caprine strain of retrovirus can productively infect only goat cells [11] and by extension cause disease in goats and not English sheep. Whether different breeds also confer varied susceptibility on different organ systems is unknown. In the United States, interstitial pneumonia seems to be the primary pathologic manifestation of infection with these retroviruses [1]. In the case reported here, the severe lesions of both the central nervous system and lungs suggest a causative strain of virus with dual tropism. Further studies with this virus are underway in Corriedale and other breeds of sheep. It is hoped the pathogenic potential of this agent and the effects of breed on expression of disease will be clarified.

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References


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