Juvenile-onset Neuronal Ceroid–Lipofuscinosis in Rambouillet Sheep


College of Veterinary Medicine, Texas A&M University, College Station, TX (JFE, RWS, JRJ); and Texas Agricultural Experiment Station, San Angelo, TX (JMS, CSM)

Abstract. Two, 8-month-old Rambouillet half-sister ewes with signs of visual loss and decreased mentation were examined. Ewe No. 1 was necropsied at 10 months of age, and after being held under observation for a further 6 months, ewe No. 2 was necropsied at 16 months of age. At that time, the ewe was blind and severely depressed. Both ewes had deposition of an autofluorescent lipopigment, identified as ceroid–lipofuscin, in neurons of the brain, spinal cord, eye, and dorsal root ganglia. The disease process was progressive and characterized by deposition of lipopigment with neuronal degeneration and severe fibrillary astrogliosis. This progressive loss of neurons in the older ewe led to severe retinal degeneration. No pigment was observed in cells outside of the nervous system and eye. Controlled breeding studies have shown that this disease has an autosomal, recessive inheritance. The disease referred to here as juvenile-onset neuronal ceroid–lipofuscinosis of Rambouillet sheep is unlike the majority of the hereditary ceroid–lipofuscinoses that occur in human beings and animals in that only the nervous system is affected. Therefore, this disease could serve as an excellent model for the study of lipopigment deposition that affects the nervous system as a result of various disease states and during aging.

Key words: Blindness; inherited disease; neuronal ceroid–lipofuscinosis; Rambouillet sheep.

Lipopigments are cytoplasmic pigments that have an affinity for neutral lipid stains. They are classified as those that occur normally in tissues (lipofuscin) and those generated experimentally or associated with pathologic conditions (ceroid). Lipofuscin is a dark brown, autofluorescent pigment that is observed within cytosomes of cells and accumulates during the normal aging process. Typically, lipofuscin stains well with oil red O, Sudan black, acid-fast, and periodic acid-Schiff stains. Although it contains a large proportion of lipid, lipofuscin is 50% protein by weight, and it is enriched in aluminum, copper, and iron. Ultrastructurally, it is observed in membrane-bound, cytoplasmic structures, presumably lysosomes, and is characterized by having polymorphic, curvilinear, or fingerprint profiles of membranes that sandwich material of variable electron density. Although most damaged cell components are metabolized and reused by cells, lipopigments may accumulate in lysosomes in a nonmetabolizable form perhaps as the enzymatic capacity of senile cells declines. Ceroid has similar staining and morphologic features to those of lipofuscin. Its accumulation in the cytoplasm is often the result of excess production of cell breakdown products that result from epigenetic factors that increase cell degeneration or that inhibit metabolism. Hereditary defects in metabolism also can give rise to lipofuscin or ceroidlike accumulations in cells. Although ceroid and lipofuscin are morphologically similar, a wide range of conditions give rise to the accumulation of these pigments in cells; therefore, they may vary biochemically. The name ceroid–lipofuscin is used by many to describe the lipopigment in hereditary disease conditions affecting several species. Ceroid–lipofuscinosis was first used to describe the lipopigment in a group of inherited storage diseases of human beings originally and incorrectly classified with the gangliosidoses. The use of this term is in recognition of the abnormal process leading to the accumulation of lipopigment in cells in these diseases and the morphologic similarity of the two lipopigments, lipofuscin and ceroid.

Neuronal ceroid–lipofuscinosis (NCL) is a documented hereditary condition that affects numerous species. NCL in the English Setter dog and in South Hampshire–Southdown crossbred sheep has been used as animal models of the human condition. In human beings, the disease has several manifestations that primarily vary in terms of the age at onset and rapidity of onset of clinical signs; however, these variations share common histopathologic lesions. Clinically, both the human and animal disease syndromes are characterized by a progressive onset of blindness, motor sensory deterioration, and mental retrogression. Histopathologically, this group of diseases is charac-
characterized by an accumulation of an autofluorescent lipopigment, ceroid–lipofuscin, primarily in neurons but also in cells of various visceral and somatic tissues. Although the lipopigment is deposited in many tissues outside of the nervous system, clinical dysfunction generally is referable to the central nervous system. A form of NCL that has lipopigment deposition only in neurons has been reported in Nubian goats with a history of progressive ataxia and paresis. Lesions of hereditary NCL with a juvenile onset in sheep from an inbred Rambouillet flock are reported here.

Case History

Two 10-month-old ewe lambs were donated to the Texas A&M University Veterinary Medical Center (TAMU-VMC) with a primary complaint of a visual deficit. Vision loss had been noted in these two range lambs on the ranch when they were 8 months old. The flock had been brought in from pasture, and when these ewes were put in any new environment, they collided with fences, gates, and feed troughs. The lambs were otherwise normal, and after a period of adjustment, they would move about cautiously in a pen and no longer collide with structures. Fundoscopic examination by the referring veterinarian revealed no abnormalities. The animals were treated with vitamin A and thiamin by the referring veterinarian and maintained in a hospital pen for observation. There was no response to treatment. Data from the flock’s breeding records showed that these lambs were half-sisters sired by a young ram that had been found dead and tangled in a fence after his first breeding season.

Upon presentation to the TAMU-VMC, the lambs were in good body condition. Ophthalmologic examination revealed slow and consensual direct pupillary responses. Mild hyperreflectivity of the tapetal fundus was noted, and the blood vessels of the fundus were judged to be smaller than normal. Although both ewes had reduced vision, they were able to work their way through a maze of obstacles; therefore, they were not considered clinically blind. The animals were judged to have decreased mentation because they had lost their herding instinct and would stand off by themselves for long periods of time. When clinically compared with other blind animals of various species, they were judged to respond slowly to auditory stimuli. The lambs were euthanatized, one (ewe No. 1) at 10 months of age and the other (ewe No. 2) at 16 months of age. The brain, both eyes, and the spinal cord as well as samples of liver, spleen, adrenal gland, thyroid, pituitary gland, lung, heart, thymus, small intestine, colon, uterus, ovary, kidney, skeletal muscle, bone marrow, mesenteric lymph node, tracheal bronchial lymph node, and submandibular lymph node were fixed in 10% neutral-buffered formalin and processed for paraffin-embedded histologic sectioning. The brains were histologically examined at the levels corresponding to plates 2, 4, 6, 10, 12, 14, and 16.
according to de Lahuntal and at the level of the frontal cortex. The spinal cords were histologically examined at the level of spinal nerves C5, C7, T3, T11, L1, L4, and S2. Histologic stains used included hematoxylin and eosin, periodic acid-Schiff (PAS), Sudan black B, Kluver-Barrera luxol fast blue, Ziehl-Nielsen acid-fast, and Holme's silver nitrate stains.

At necropsy, abnormalities of ewe No. 1 were noted only in the brain. The sulci of the cerebral hemispheres were accentuated, and the gyri were narrowed. There was a reduction in size of the cerebral hemispheres that resulted in an increased space between the brain and the cranial cap. Because of the reduction in amount of cortical tissue, the dorsal nerve root was narrowed, and the gyri were narrowed. There was a reduction in amount of cortical tissue and in the brain stem, there was a mild and symmetric widening of the lateral ventricles (hydrocephalus ex vacuo) mesencephalic aqueduct, and fourth ventricle. The cerebellum was grossly normal.

Histologic lesions were detected in neurons of the central nervous system, dorsal root ganglia, and retina. There was a decreased number of cortical neurons of the cerebrum and atrophy of the entire cortex (Fig. 1). Neurons in the cerebral cortex, brain stem, spinal cord, dorsal root ganglia, and eye contained granules of various sizes that appeared hyalinized and eosinophilic when stained with hematoxylin and eosin, and the material in these granules fluoresced pale yellow or green when viewed with ultraviolet light. This material accumulated in cells in smooth-edged round or oval granules that varied in size from 1 to 7 μm and stained positively with PAS, Sudan black B, Kluver-Barrera luxol fast blue, and Ziehl-Nielsen acid-fast stains (Fig. 2) and was compatible in appearance with the lipopigment referred to as ceroid-lipofuscin. In the retina, there was mild thinning of the rod and cone layer, and pigment granules were seen in photoreceptor cell bodies. The ganglion cells of the retina also contained lipopigment. The outer nuclear layer was slightly thinned. The neurons of the autonomic nervous system (neurons of the dorsal vagal nucleus and of Meissner's and Auerbach's plexes) in the viscera. Neurons of the dorsal autonomic neurons of terminal ganglia (Meissner's and Auerbach's plexes) in the viscera. Neurons of the dorsal nucleus of the vagus nerve and intermediate gray matter of the thoracic and sacral regions of the spinal cord of ewe No. 2 contained lipopigment. No sympathetic autonomic ganglia were available for examination.

Ewe No. 2 was euthanatized at 16 months of age, and tissues were collected for histologic examination to gain insight into lesion progression. Between 10 and 16 months of age, the animal became more depressed and would spend long periods staring at the ceiling. An electroretinogram was performed on this ewe just prior to necropsy, and she showed no response to photostimuli. A hemogram done prior to necropsy showed no abnormalities or lipopigment in leukocytes. At necropsy, this ewe was slightly small for her age and breed. Her cerebral hemispheres were markedly flattened dorsoventrally with deep sulci and narrowed gyri (Figs. 3, 4). The ventricles were three times normal size. The brain weighed 59 g (a control brain weighed 121 g) and was firmer than normal. The cerebellum was mildly reduced in size. Microscopically, lesions were similar to those seen in ewe No. 1. Although more severe (Fig. 5). All examined neurons in sections of the brain, spinal cord, and spinal dorsal root ganglia contained autofluorescent lipopigment. Cerebral cortical neurons were markedly reduced in number, and remaining neurons contained lipopigment. Small degenerating neurons whose cytoplasm was totally occupied by granules of lipopigment frequently were observed (Fig. 6). The cerebral cortical neuropil contained few axons. Reactive astrocytes were increased in number, and fibrillary astrogliosis was prominent in the cortex. In the retina, there was severe atrophy of all layers, with granules of lipopigment present in remaining neurons of the ganglionic cell layer and in the atrophic photoreceptor layer (Figs. 7, 8). Traces of lipopigment deposition also were detected in the cells of the inner nuclear layer. Although some lipopigment was noted in Purkinje cells in the cerebellum, there was only a mild reduction in number of these neurons. No lipopigment was present in post-ganglionic autonomic neurons of terminal ganglia (Meissner's and Auerbach's plexes) in the viscera. Neurons of the dorsal nucleus of the vagus nerve and intermediate gray matter of the thoracic and sacral regions of the spinal cord of ewe No. 2 contained lipopigment. No sympathetic autonomic ganglia were available for examination.

Discussion

Lipopigment may accumulate in cells of many tissues as a result of inherited storage diseases, metabolic derangements, and the normal aging process. Regarding the ceroid-lipofuscinoses, it is controversial as to whether these pigments are formed by a single process. Originally, these lipopigments were thought to accumulate as a result of abnormalities in the peroxidation of lipids. However, there are several hereditary human subtypes of neuronal ceroid-lipofuscinosis (NCL) that have eponymous designations for infantile, late infantile, juvenile, or adult onset of signs, and the variety of clinical manifestations suggests there may be a heterogeneity in metabolic derangements. Evidence exists from the study of both human and animal
Fig. 3. Brain; ewe No. 2, a 16-month-old Rambouillet with neuronal ceroid-lipofuscinosis. The cerebral hemispheres are atrophic with narrow gyri and prominent, deep sulci. The cerebellum is only mildly flattened. Bar = 1 cm.

Fig. 4. Brain; ewe No. 2, a 16-month-old Rambouillet with neuronal ceroid-lipofuscinosis. There is dorsoventral flattening of the atrophic cerebrum and dilation of the lateral ventricle and mesencephalic aqueduct (arrow). Bar = 1 cm.

hereditary ceroid-lipofuscinoses that precursors of these lipopigments include both lipids and proteins. The presence of a protein component associated with the membrane structures visualized ultrastructurally is thought to account for the electron-dense appearance of the material seen in inclusions of affected sheep and human beings with NCL. Current analysis by other workers of the abnormal protein accumulating in lysosomes in NCL of Southdown-cross sheep and in the late-infantile, juvenile, and adult forms of human NCL indicates that this protein is derived from the N-terminal portion of the lipid-binding subunit of mitochondrial ATP synthase. Although luxol fast blue is a lipid stain, the researchers studying the South-
Edwards, Storts, Joyce, Shelton, and Menzies

Vet Pathol 31:1, 1994

Fig. 5. Cerebral cortex; ewe No. 2, a 16-month-old Rambouillet with neuronal ceroid-lipofuscinosis. There is severe neuronal loss and an astrogliosis. HE. Bar = 100 μm.

down-cross sheep model of NCL hypothesized that the strong affinity of ceroid-lipofuscin for this stain was due to the protein component of the pigment. The term proteolipid proteinosis has been proposed as a better descriptor of these inherited diseases, especially because the pathogenesis of the conditions is undetermined. The group may represent several different biochemical defects; however, until the defects are identified, it is prudent to use the generic term, ceroid-lipofuscinosis, to characterize the condition and avoid confusion.

The prominent astrogliosis detected in the affected Rambouillet sheep encountered in this study has also been reported to occur in Southdown-cross sheep with NCL. Although this change is not often emphasized

Fig. 6. Cerebral cortex; ewe No. 2, a 16-month-old Rambouillet with neuronal ceroid-lipofuscinosis. There is marked loss of neurons and a fibrillary astrogliosis. Broad arrows indicate hyperplastic astroglial fibers. Remaining neurons contain dense accumulations of lipopigment (thin arrows). HE. Bar = 30 μm.
Neuronal Ceroid-Lipofuscinosis in Sheep

Fig. 7. Retina; ewe No. 1, a 10-month-old Rambouillet with neuronal ceroid-lipofuscinosis. There is minimal thinning of the layer of rods and cones and the outer nuclear layer. HE. Bar = 30 μm.

Fig. 8. Retina; ewe No. 2, a 16-month-old Rambouillet with neuronal ceroid-lipofuscinosis. When compared with the retinal lesions seen at 10 months of age in ewe No. 1, there has been a reduction in the thickness of the fiber layer, inner plexiform layer, outer plexiform layer, and outer nuclear layer. The photoreceptor layer is nearly devoid of rods and cones. HE. Bar = 30 μm.

In other reports of ceroid-lipofuscinosis in animals and human beings, astrocytic proliferation accompanies neuronal loss in a variety of diseases of the central nervous system. The mechanisms responsible for this response have not been fully determined; however, recent studies have shown that neurons can directly influence astrocytic proliferation, usually in an inhibitory fashion. Therefore, loss of neurons as is seen in NCL would be expected to allow astrocytes to proliferate.

A possible toxic etiology was considered in the affected Texas flock, but no toxic plants or sources of exogenous toxins were identified. A neuronal ceroid-lipofuscinosis-like condition in horses and sheep grazing *Trachyandra divaricata* (branched onion weed) in Australia and South Africa has been reported. Unlike NCL in Rambouillet sheep, the condition produced by this plant involves the deposition of lipopigment in neurons of the Meissner's and Auerbach's plexes and in Kupffer cells of the liver.

In instances where the inheritance pattern is known, NCL in human beings and animals is a condition associated nearly always with an autosomal recessive inheritance. The occurrence of cases in related Rambouillet sheep was an early observation that suggested NCL in these animals was a heritable disease. NCL has also been recognized in three other flocks of Rambouillet sheep in Texas, and the results of an investigation of one of these flocks were included in a brief report. Clinical signs and lesions were identical to those of the two sheep reported here except that no ocular lesions were described. Unfortunately, no breeding history was available regarding those affected range animals. In the flock of origin of the two sheep in the present report, there had been some degree of inbreeding, and reexamination of the flock revealed three other animals with similar signs, two daughters and a son of the same ram that sired the ewes in this study. Other flocks using rams related to that sire also have had cases of blind sheep. A controlled breeding trial using known Rambouillet carriers or likely carriers (based on pedigree data) related to the ewes of this study was performed, and an autosomal recessive mode of inheritance has been established for this condition.

The clinical manifestation of Rambouillet NCL is similar to that of NCL reported in human beings and other animals. NCL is characterized by an insidious onset of a visual deficit and loss of mentation. The signs are progressive and eventually result in total blindness and severely depressed mentation. Temporally, the signs are associated with progressive accumulation of ceroid-lipofuscin in neurons and several other tissues. In affected human beings and South Hampshire sheep, this process begins in the fetus, and biopsy of human fetal trophoblasts that contain pigment has permitted prenatal diagnosis. The accumulation of ceroid-lipofuscin with loss of neuronal function and neuronal death would account for the signs seen in the sheep examined in the present study. Blindness was due to neuronal degeneration in the retina and in areas of the brain controlling visual perception (e.g., the lateral geniculate body and visual cortex of the occipital lobe), as is reported for NCL in human beings and other animals.

No ceroid-lipofuscin was noted in the visceral or somatic tissues of the two sheep in this study. Lipo-
pigment may have appeared at a later age; however, the sheep euthanized at 16 months of age was already in the advanced stages of the disease. The absence of ceroid–lipofuscin in the dorsal ganglion of the vagus in ewe No. 1 indicated that the general or special visceral efferent neurons may be affected differentially in this disease. The suggestion of differential involvement would be supported by the absence of ceroid–lipofuscin in visceral autonomic ganglia of the intestines of both animals examined. However, autonomic neurons of the vagal nucleus and the autonomic tracts (intermediate gray columns) in the thoracic and sacral spinal cords of ewe No. 2 contained lipopigment, indicating that the autonomic nervous system is affected to some degree. Future studies should address the distribution of ceroid–lipofuscin in the autonomic nervous system.

Recent efforts to improve the Rambouillet breed through a ram testing and selection program apparently facilitated the emergence of NCL. The autosomal recessive heredibility of this disease has been confirmed with controlled-breeding data. Because pigment deposition is confined to neurons in NCL of the sheep, the biochemical origin of this form of ceroid–lipofuscinosis appears to be different from those of the human and animal models of NCL. Controlled breeding of affected relatives has provided subjects for biochemical studies, and future studies of the biochemical defect are needed to characterize NCL in Rambouillet sheep. This information will help elucidate the pathways that are involved in the formation and metabolism of lipofuscin and ceroid lipopigments.

References


Request reprints from Dr. J. Edwards, Department Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4463 (USA).