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Feline Niemann-Pick Disease Type C

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Biological Features

Human Niemann–Pick disease type C (NPC) is an autosomal recessive neurovisceral lysosomal storage disorder in which cholesterol lipidosis results from defective intracellular transport of unesterified cholesterol.[1] Unlike types A and B, NPC is not a primary sphingomyelinase deficiency.[2,3] The primary molecular defect of NPC is unknown, however, NPC in humans has recently been linked to chromosome 18.[4] The regulatory mechanisms of cholesterol metabolism have been shown to be impaired, resulting in retarded esterification of exogenous cholesterol with accumulation of unesterified cholesterol in lysosomes.[1,5] NPC is a complex lipidosis with storage of unesterified cholesterol, glycolipids, and phospholipids including sphingomyelin. The disease, with neurological deterioration as its central characteristic, often becomes evident at an early age and usually results in delayed mental and motor development and premature death. The disease in humans is heterogeneous clinically, with an infantile (acute), juvenile (subacute), and, rarely, adult (chronic) onset expression.[6] Early onset of symptoms has been associated with higher mortality.[7] There is currently no effective treatment for NPC.

Animal Model

A 9-week-old domestic short-hair kitten with neurological signs including progressive ataxia, head, and whole body intention tremor, and dysmetria was identified. Neurovisceral histopathological lesions were consistent with a lysosomal storage disease. Clinical, biochemical, and morphological findings resembled those of sphingolipidosis similar to human NPC.[8] The dam and littermates were donated to Colorado State University, and a breeding colony has been established. We report here on the clinical, biochemical, and morphological findings in 26 affected cats.

Clinical neurological manifestations of the disease have been reported[9] and include onset of head and whole body intention tremor at 8 to 12 weeks of age, with rapid progression to severe dysmetria and ataxia and death by 8 to 10 months of age. Low birth weight and hepatomegaly are consistent findings in affected cats. Liver weights of affected cats average fivefold that of normal cats. There is no progressive difference in liver weight as a percentage of body weight with age. Brain weights of affected cats are approximately twofold heavier than normal cats, presumably due to storage.

Serum biochemical abnormalities include increased liver alkaline phosphatase activity, approximately fourfold above normal controls. Serum alkaline phosphatase activity is consistently increased in 2-week-old kittens and is used as a reliable early indicator of affected status. Other serum biochemical abnormalities include increases in alanine aminotransferase activity, aspartate aminotransferase activity, bile acids, and mild increases in total bilirubin as the disease progresses. All of these serum biochemical changes are referrable to hepatic disease. None of the affected cats have been jaundiced or shown evidence of liver failure.

Liver lipid extract by the method of Folch et al[10] with subsequent analysis by thin layer chromatography using standard methods[11] reveals a complex lipid storage pattern with abnormal accumulation of unesterified cholesterol, sphingomyelin, and glycolip-

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ids, including glucosylceramide and lactosylceramide. Unesterified cholesterol levels in liver and spleen are significantly higher in affected cats compared to normal controls. Cholesterol ester levels are significantly lower in liver and spleen of affected cats in comparison to normal controls. Thin layer chromatographic analysis of brain tissue extract[11] reveals accumulation of GM2 and GM3 gangliosides.

Cultured fibroblasts of affected cats have decreased ability to esterify exogenous cholesterol (method previously described).[8,12,13] Fibroblasts demonstrate a perinuclear accumulation of unesterified cholesterol following cholesterol depletion and subsequent challenge with exogenous cholesterol in the form of low-density lipoprotein. This unesterified cholesterol accumulation is demonstrated by strongly positive staining with the fluorescent polyene antibiotic filipin that binds specifically to unesterified cholesterol (Figure 1).

Cytology of bone marrow and liver aspirates of young kittens demonstrate the storage process and aid in diagnosis before the onset of clinical signs. Foamy macrophages or sea-blue histiocytes[14] are infiltrated throughout the bone marrow (Figure 2) and increase in number with age. Hepatocytes and Kupffer cells are enlarged with foamy cytoplasm. The hepatocytes stain positive for cholesterol with Schultz and filipin stains. Hepatocytes are negative to slightly positive on periodic acid-Schiff with diastase staining. Kupffer cells are periodic acid-Schiff-positive and remain positive upon the addition of diastase. The use of periodic acid-Schiff staining allows the Kupffer cells to be readily distinguished from glycogen-rich liver cells. Vacuolated lymphocytes are seen on Wright–Giemsa-stained blood films and increase in number and degree of vacuolation with age.

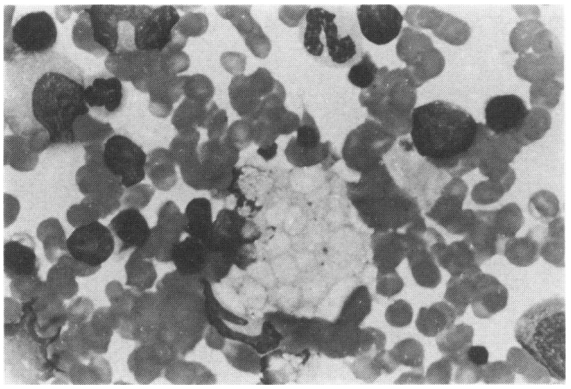

Figure 2. *Foamy macrophage in bone marrow aspirate from NPC-affected cat (Wright's–Giemsa, ×660).*

Light and electron microscopic evaluation of tissues collected at necropsy from one cat of this colony have been described.[8,9] A neurovisceral storage process is evidenced by cytoplasmic vacuolation, representative of lysosomes distended with storage material. Substrate accumulation is present in neurons throughout the central nervous system and retinal ganglion cells as evidenced by pale, foamy cytoplasm of neurons with displacement of nuclei and Nissl substance. Cerebellar Purkinje cells are similarly affected with progressive cellular degeneration and death (Figure 3). Another feature of the neurological disease process is the presence of axonal spheroids predominating in GABAergic neurons (Walkley et al, unpublished data) and ectopic dendrite growth on cortical pyramidal neurons.[15] Visceral storage is evident in the liver, spleen (Figure 4), lung, gut-associated lymphoid tissue, and lymph nodes. Foamy macrophages are present in each of these visceral organs. Hepatocytes are pale, swollen, and hepatic architecture is markedly disrupted (Figure 5).

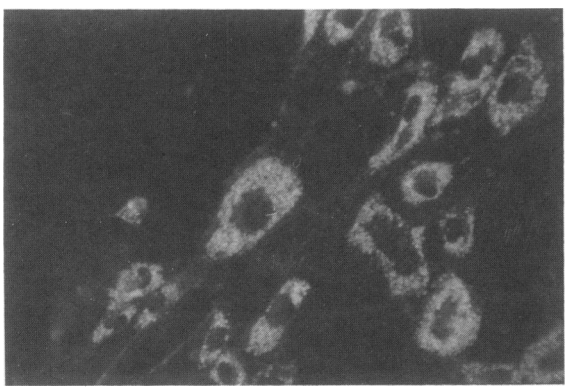

Figure 1. *Feline NPC cultured skin fibroblasts grown in lipoprotein deficient medium, incubated with low density lipoprotein (50 μg/ml) for 24 hours, then stained for unesterified cholesterol with filipin (×128).*

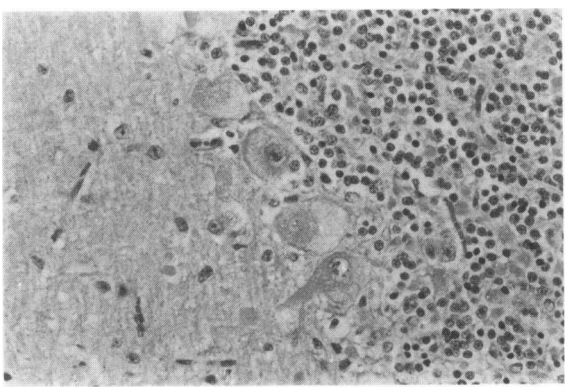

Figure 3. *Purkinje cells from the cerebellum of a 5-month-old NPC-affected cat, demonstrating vacuolated cytoplasm, displaced Nissl substance and nuclei (H&E, ×200).*

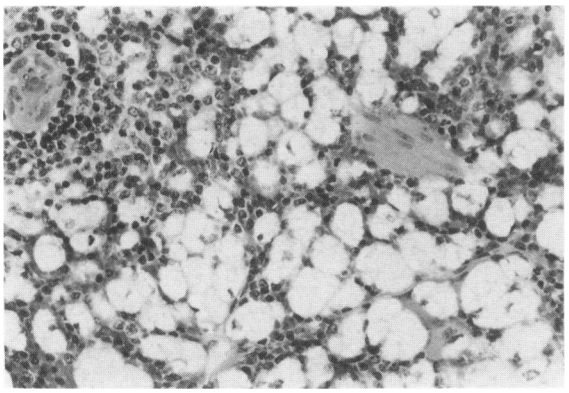

Figure 4. *Feline NPC spleen with lipid-laden macrophages throughout red pulp (H&E, ×200).*

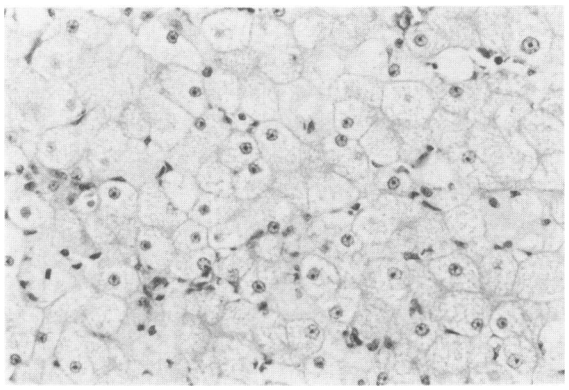

Figure 5. *Liver of NPC cat with swollen pale hepatocytes and vacuolated cytoplasm (H&E, ×200).*

Comparison with Human Disease

Similarities between this feline lipid storage disorder and human NPC include onset and progression of clinical neurological signs, autosomal recessive inheritance, neurovisceral storage pattern as evidenced by light microscopy, and biochemistry of storage material consisting of unesterified cholesterol, sphingomyelin and other phospholipids, glycolipids, and gangliosides in the central nervous system. Impaired ability of cultured skin fibroblasts to esterify exogenous cholesterol with perinuclear accumulation of unesterified cholesterol demonstrated by filipin staining are identical to the human disease. The clinical heterogeneity of human NPC has been described.[3,6,7] The age of onset and progression of clinical disease of this animal model are most consistent with that of group IIS Niemann–Pick disease, which is nonsphingomyelinase-deficient with subacute onset.[6]

Usefulness of the Model

Studies utilizing this feline model of group IIS NPC may contribute to a better understanding of this specific lysosomal storage disorder and cholesterol metabolism. Investigations into the pathogenetic mechanisms and modes of therapy for NPC in humans are limited by ethical constraints. These cats will provide a domestic animal model for investigation of treatment modalities and in depth clinical, pathological, and biochemical studies, thus providing information directly pertinent to the pathogenesis of the disorder in particular, and cholesterol metabolism in general. Therapeutic modality investigations in progress include ultra-low cholesterol diet studies, cholesterol-lowering drug studies, and allogeneic bone marrow transplantation.[16]

Availability

This breeding colony was established in 1989. Of 77 kittens produced to date from obligate heterozygote breedings, 26 (33%) have been NPC-affected. Six female and two male obligate heterozygotes constitute the breeding stock. The colony has been outbred in an effort to decrease neonatal mortality attributed to inbreeding. Limited numbers of affected kittens are available.

Acknowledgment

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